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 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  DEC 21  IPC search and display fields enhanced in CA/CAplus with the
                  IPC reform
 NEWS  4  DEC 23  New IPC8 SEARCH, DISPLAY, and SELECT fields in USPATFULL/
                  USPAT2
 NEWS  5  JAN 13  IPC 8 searching in IFIPAT, IFIUDB, and IFICDB
 NEWS  6  JAN 13  New IPC 8 SEARCH, DISPLAY, and SELECT enhancements added to
                  INPADOC
 NEWS  7  JAN 17  Pre-1988 INPI data added to MARPAT
 NEWS  8  JAN 17  IPC 8 in the WPI family of databases including WPIFV
 NEWS  9  JAN 30  Saved answer limit increased
 NEWS 10  JAN 31  Monthly current-awareness alert (SDI) frequency
                  added to TULSA
 NEWS 11  FEB 21  STN AnaVist, Version 1.1, lets you share your STN AnaVist
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 NEWS 12  FEB 22  Status of current WO (PCT) information on STN
 NEWS 13  FEB 22  The IPC thesaurus added to additional patent databases on STN
 NEWS 14  FEB 22  Updates in EPFULL; IPC 8 enhancements added
 NEWS 15  FEB 27  New STN AnaVist pricing effective March 1, 2006
 NEWS 16  FEB 28  MEDLINE/LMEDLINE reload improves functionality
 NEWS 17  FEB 28  TOXCENTER reloaded with enhancements
 NEWS 18  FEB 28  REGISTRY/ZREGISTRY enhanced with more experimental spectral
                  property data
 NEWS 19  MAR 01  INSPEC reloaded and enhanced
 NEWS 20  MAR 03  Updates in PATDPA; addition of IPC 8 data without attributes
 NEWS 21  MAR 08  X.25 communication option no longer available after June 2006

 NEWS EXPRESS  FEBRUARY 15 CURRENT VERSION FOR WINDOWS IS V8.01a,
               CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 19 DECEMBER 2005.
               V8.0 AND V8.01 USERS CAN OBTAIN THE UPGRADE TO V8.01a AT
               http://download.cas.org/express/v8.0-Discover/

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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 23:13:49 ON 19 MAR 2006

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY.   SESSION
FULL ESTIMATED COST                                      0.21      0.21

FILE 'USPATFULL' ENTERED AT 23:14:08 ON 19 MAR 2006
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 16 Mar 2006 (20060316/PD)
FILE LAST UPDATED: 16 Mar 2006 (20060316/ED)
HIGHEST GRANTED PATENT NUMBER: US7013485
HIGHEST APPLICATION PUBLICATION NUMBER: US2006059596
CA INDEXING IS CURRENT THROUGH 16 Mar 2006 (20060316/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 16 Mar 2006 (20060316/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2005


=> e humeau laurent/in
E1            1     HUME WYATT R/IN
E2            7     HUMEAU DOMINIQUE/IN
E3            6 --> HUMEAU LAURENT/IN
E4            1     HUMEAU VINCENT/IN
E5            1     HUMECKI MICHAEL G/IN
E6            1     HUMEL JOSEPH M/IN
E7            7     HUMELE HEINZ/IN
E8            7     HUMELSINE BILLY MACK/IN
E9            1     HUMELSINE JAMES E/IN
E10           1     HUMEN ANDREW JR/IN
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E12          1     HUMEN JOHN/IN

=> s e3
L1           6 "HUMEAU LAURENT"/IN

=> d l1,ti,1-6

L1   ANSWER 1 OF 6  USPATFULL on STN
TI     Vector packaging cell line

L1   ANSWER 2 OF 6  USPATFULL on STN
TI     Increased transduction using ABC transporter substrates and/or
       inhibitors

L1   ANSWER 3 OF 6  USPATFULL on STN
TI     Methods for stable transduction of cells with viral vectors

L1   ANSWER 4 OF 6  USPATFULL on STN
TI     Conditionally replicating vectors for inhibiting viral infections

L1   ANSWER 5 OF 6  USPATFULL on STN
TI     Methods for stable transduction of cells with hiv-derived viral vectors

L1   ANSWER 6 OF 6  USPATFULL on STN
TI     Conditionally replicating vectors for inhibiting viral infections


=> d l1,cbib,ab,clm,1-6

L1   ANSWER 1 OF 6  USPATFULL on STN
2006:3995 Vector packaging cell line.
    Humeau, Laurent, Germantown, MD, UNITED STATES
    Slepushkin, Vladimir, Damascus, MD, UNITED STATES
    Paszkiet, Brian, Frederick, MD, UNITED STATES
    Ni, Yajin, Germantown, MD, UNITED STATES
    VIRxSYS Corporation, Gaithersburg, MD, UNITED STATES (U.S. corporation)
    US 2006003452 A1 20060105
    APPLICATION: US 2005-172147  A1  20050630 (11)
    PRIORITY: US 2004-585464P  20040701 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The invention relates to a method of increasing vector transduction in
       target cells. The invention provides for the recombinant engineering of
       a packaging cell line to be capable of expressing one or more membrane
       proteins which facilitate binding to, and activation of, a target cell.
       The invention also provides for recombinant engineering of a cell that
       endogenously expresses one or more such membrane proteins into a
       packaging cell line. A vector packaged into viral particles via use of
       such cell lines would comprise an outer envelope containing these
       proteins. The particles would be specifically suited for binding and
       targeting to a target cell to facilitate transduction thereof with the
       vector. The target cell may also be simultaneously activated
       (stimulated) by the packaged vector in the absence of exogenously
       supplied stimulatory molecules.
CLM    What is claimed is:
       1. A recombinant retroviral packaging cell comprising a first nucleic
       acid molecule capable of expressing, in said packaging cell, at least
       one membrane associated non-viral ligand which binds a cell surface
       molecule of a target cell,  wherein said cell produces no viral
       particles in the absence of a second nucleic acid molecule.

       2. The cell according to claim 1 wherein said retroviral packaging cell
       comprises  i) a heterologous nucleic acid molecule capable of expressing
       a viral ligand which binds a cell surface molecule of a target cell and
       which optionally functions to mediate fusion of a cell membrane
       containing said viral ligand with said target cell; or  ii) a
       heterologous nucleic acid molecule which is capable of expressing CD49d,
       CD54, CD80, CD86, or a combination thereof.

       3. The cell according to claim 1 wherein said target cell is an antigen
       presenting cell (APC), a cell of the hematopoietic lineage, a stem cell,
       a hematopoietic stem cell, a lymphocyte, a neuron, an endothelial cell,
       or a tumor cell.

       4. The cell according to claim 1 wherein said at least one membrane
       associated non-viral ligand(s) is  i) a co-stimulatory molecule that
       binds a T cell surface molecule to activate T cell proliferation when
       the CD3/TCR complex of said T cell is bound by a natural or artificial
       ligand or by a specific Ab, and/or  ii) a molecule that plays a role in
       cell-cell adhesion via binding to said cell surface molecule.

       5. The cell according to claim 1 wherein  i) said at least one membrane
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after binding said cell surface molecule; ii) said at least one membrane associated non-viral ligand(s) comprise two such ligands; iii) said membrane associated non-viral ligand(s) is that of a hematopoietic cell; iv) said membrane associated non-viral ligand(s) are transmembrane protein(s); or v) said membrane associated non-viral ligand(s) is CD28 or CD28BP or CD40 or CD62L or CD80 (B7-1) or CD86 (B7-2) or Fas ligand (FasL) or CD70 or LFA-3 (CD58) or B7-H1 (PD-L1) or B7-H2 or B7-H3 (B7RP-2) or B7-H4 or CD2 or CD3 or CD3/TCR complex or CD11a or CD26 or CD27 or CD28 or CD30L or CD32 or CD38 or CD40L (CD154) or CD45 or CD49 or CD50 (ICAM-3) or CD54 (ICAM-1) or CD100 or CD122 or CD137L (4-1BB Ligand) or CD153 or CTLA-4 (CD152) or ICOS or OX40L (CD134) or PD-1 or PD-L2 (B7-DC) or SLAM (CD150) or TIM-1 or TIM-2 or TIM-3 or TIM-4 or 2B4 (CD244), or a combination thereof.

6. The cell according to claim 1 wherein i) said membrane associated non-viral ligand(s) is a B7 related molecule such as $B7\text{-}H_1$, $B7\text{-}H_2$ (also known as ICOS-L, B7RP-1, and GL50), $B7\text{-}H_3$, and $B7\text{-}H_4$; ii) said membrane associated non-viral ligand(s) binds LFA-1, or a complex of CD11a with CD18, such as ICAM-2 (CD102), ICAM-3 (CD50), ICAM-4 (LW), or ICAM-5 (telencephalin); or iii) said membrane associated non-viral ligand(s) is a PD-1 ligand, such as $PD\text{-}L_2$ or $PD\text{-}L_1$; an OX40 ligand (CD154) which binds OX40 (CD134); a 4-1BB ligand which binds 4-1BB (CD137); a ligand which binds LFA-2 (CD2), such as CD15, CD48, CD58, or CD59; a ligand which binds CD5, such as CD72; or a ligand which binds LFA-3 (CD58), such as CD2; iv) said membrane associated non-viral ligand(s) is an antibody or antibody fragment that binds LFA-1, CD18, CD11b, CD11c, CD11d or CD43; or v) said membrane associated non-viral ligand(s) is a microbial protein that binds LFA-1, CD18, CD11b, CD11c, CD11d or CD43.

7. The cell according to claim 2, wherein said retroviral packaging cell comprises a heterologous nucleic acid molecule which is capable of expressing the polio virus receptor CD155, the wild type herpes simplex virus (HSV)-1 envelope protein, or another viral envelope protein; and i) the target cell is a hematopoietic stem cell; ii) the target cell is CD34+, CD33+, CD14+, or expresses nectin 1; iii) the target cell is a T cell; iv) the target cell is a dendritic cell or a B cell in the germinal center of a lymph node; v) the target cell is a fibroblast.

8. A method of producing a recombinant retroviral packaging cell according to claim 1, said method comprising introducing, into a cell, a nucleic acid molecule capable of expressing, in said packaging cell, a viral gene product necessary for packaging and/or replication of a retrovirus, and at least one nucleic acid molecule capable of expressing, in said packaging cell, at least one membrane associated non-viral ligand(s) which bind a cell surface molecule of a target cell.

9. A method of packaging a viral vector, said method comprising introducing a second nucleic acid molecule encoding or comprising a retroviral vector into a recombinant cell according to claim 1, and culturing said cell under conditions wherein said cell packages said vector into a particle comprising said membrane associated non-viral ligand(s) which bind a cell surface molecule of a target cell.

10. A retroviral vector packaging cell comprising a cell that endogenously expresses at least one membrane associated ligand capable of binding to a cell surface molecule of a target cell or tissue.

11. The packaging cell of claim 10 wherein the cell interacts with the target cell or tissue in vivo.

12. The packaging cell of claim 11 wherein the cell is a bone marrow stromal cell and the target cell is a hematopoietic stem cell.

13. The packaging cell of claim 10 wherein the ligand is an integrin and the target cells are CD34+ cells.

14. The packaging cell of claim 13 wherein the ligand is SDF-1, VLA-4, VLA-5, or LFA-1, and the hematopoietic stem cell is CD34+ and CD38-/CXCR4+, or CD34+ and CD38 low/CXCR4+.

15. The cell according to claim 10 wherein said retroviral packaging cell comprises a heterologous nucleic acid molecule capable of expressing a viral ligand which binds a cell surface molecule of a target cell and which optionally functions to mediate fusion of a cell membrane containing said viral ligand with said target cell.

16. The cell according to claim 1 wherein said cell is a T cell or a cell of a T cell line.

17. The cell according to claim 1 wherein said cell expresses CD86 but not CD54.

18. A recombinant retroviral packaging cell comprising a first nucleic acid molecule capable of expressing, in said packaging cell, at least one membrane associated non-viral ligand which binds a cell surface molecule of a target cell and activates or stimulates the cell into cell cycle transition.

19. The cell according to claim 18 wherein said packaging cell expresses a retroviral structural protein or accessory protein.

20. The cell according to claim 18 further comprising a retroviral vector to be packaged.

L1 ANSWER 2 OF 6 USPATFULL on STN
2005:143779 Increased transduction using ABC transporter substrates and/or inhibitors.
Davis, Brian, Gaithersburg, MD, UNITED STATES
Rumeau, Laurent, Germantown, MD, UNITED STATES
Dropulic, Boro, Ellicott City, MD, UNITED STATES
US 2005123514 A1 20050609
APPLICATION: US 2004-839118 A1 20040504 (10)
PRIORITY: US 2003-468207P 20030505 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to improvements in the ability to transduce a retroviral vector borne nucleic acid into cells expressing ABC transporters by use of a substrate and/or inhibitor of said transporter. Compositions and kits relating to the practice of the methods are also disclosed. Methods to determine the level of increased transduction provided by a substrate and/or inhibitor compound are also provided.
CLM What is claimed is:
1. A method for increasing the transduction of an ABC transporter expressing cell with a retroviral vector, said method comprising a) contacting said cell with a compound that is a substrate of said transporter; and b) contacting said cell with said vector.

2. The method of claim 1, wherein said retroviral vector is a lantiviral vector.

3. The method of claim 1, wherein said compound is both a substrate and an inhibitor of said transporter.

4. The method of claim 2, wherein said compound is both a substrate and an inhibitor of said transporter.

5. The method of any one of claims 1-4 wherein said cell is CD34+.

6. The method of any one of claims 1-4 wherein said cells are hematopoietic stem cells.

7. The method of any one of claims 1-4 wherein said cells are primary cells.

8. The method of claim 7 wherein said cells are from bone marrow, cord blood, or peripheral blood.

9. The method of any one of claims 1-4 wherein said compound is selected from verapamil, diltiazem, quinindine, and ritonavir.

10. The method of claim 2 or 4 wherein said lentiviral vector is derived from HIV or SIV.

11. The method of any one of claims 1-4 wherein said vector is pseudotyped with VSV-G:

12. The method of claim 1 or 3 wherein said vector is derived from an oncoretrovirus.

13. The method of claim 1 or 3 wherein said vector is derived from a Spumavirus.

14. A method of determining the level of increased transduction of a retroviral vector in a cell mediated by an ABC transporter substrate or inhibitor compound, said method comprising a) providing a population of said cell; b) contacting one or more cells of said population with said vector in the presence of said compound to produce a first contacted cell or cells and contacting a second cell or cells of said population with said vector in the absence of said compound to produce a second contacted cell or cells; and c) determining the level of transduction with said vector in said first contacted cell or cells and in said second contacted cell or cells.

15. The method of claim 14 wherein said population of cells are primary cells.

16. The method of claim 15 wherein said cells are from a human being.

17. The method of any one of claims 14-16 wherein said cell is known to express an ABC transporter inhibitor.

18. The method of claim 14 wherein said vector is a lentiviral vector.

19. The method of claim 15 wherein said vector is a lentiviral vector.

20. The method of claim 16 wherein said vector is a lentiviral vector.

21. The method of claim 17 wherein said vector is a lentiviral vector.

22. Use of a retroviral vector for the preparation of a medicament for increasing the transduction of an ABC tranporter expressing cell.

L1 ANSWER 3 OF 6 USPATFULL on STN
2004:82307 Methods for stable transduction of cells with viral vectors.
Humeau, Laurent, Germantown, MD, UNITED STATES
Han, Wei, Montgomery Village, MD, UNITED STATES
Lu, Xiaobin, Germantown, MD, UNITED STATES
Slepushkin, Vladimir, Damascus, MD, UNITED STATES
Lesher, Mechelle, Columbia, MD, UNITED STATES
Davis, Brian, Gaithersburg, MD, UNITED STATES
Chang, Yung-Nien, Cockeysville, MD, UNITED STATES
Dropulic, Boro, Ellicott City, MD, UNITED STATES
US 2004062756 A1 20040401
APPLICATION: US 2003-664331 A1 20030916 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods, as well as compositions related thereto, for the efficient transduction of cells using viral vectors. The efficiency of transduction is increased by contacting the cell to be transduced with one or more molecules that bind the cell surface. Contact with a cell surface binding molecule may occur before, after, or simultaneously with contact between the viral vector and the cell. The transduced vectors may be constructed to express a gene of interest, permitting the transduced cells to be used as therapeutic and prophylactic agents.

CLM What is claimed is:

1. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells comprising contacting the surface of said cells with both a lentiviral vector and at least one molecule which binds said cell surface wherein said contacting occurs in vitro or ex vivo and wherein greater than about 90% of the cells are stably transduced after about 14 days.

2. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs before contacting the cells with at least one cell surface binding molecule.

3. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs simultaneously with contacting the cells with at least one cell surface binding molecule.

4. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs after contacting the cells with at least one cell surface binding molecule.

5. The method of claim 1 where said contacting with a lentiviral vector occurs more than once.

6. The method of claim 1 wherein said lentiviral vector is derived from HIV-1.

7. The method of claim 1 wherein said cell surface binding molecule is an antibody, a ligand or a cell surface molecule.

8. The method of claim 1 wherein said lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, vpr, vpu, tat or rev genes.

9. The method of claim 8 wherein said sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, vpr, vpu, tat or rev genes.

10. The method of claim 1 wherein said lentiviral vector is derived from HIV-2.

11. The method of claim 1 wherein said lentiviral vector is a pseudotyped vector.

12. The method of claim 11 wherein said pseudotyped vector contains the vesicular stomatitis virus G envelope protein.

13. The method of claim 1 wherein said lentiviral vector is a chimeric vector comprising HIV-1 and HIV-2 sequences.

14. The method of claim 1 wherein said hematopoietic cell is a CD4 positive cell.

15. The method of claim 1 wherein said hematopoietic cell is a lymphocyte.

16. The method of claim 15 wherein said lymphocyte is a CD4 or CD8 positive cell.

17. The method of claim 1 wherein said hematopoietic cell is a CD34 positive cell.

18. The method of claim 17 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or antibodies that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

19. The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or antibodies that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

20. The method of claim 1 wherein the said cell is a dendritic cell or a cell capable of differentiating into a dendritic cell.

21. The method of claim 20 wherein said at least one cell surface binding molecule is selected from compositions comprising GM-CSF, IL-4, and TNF-alpha; GM-CSF and interferon-alpha; or antibodies that are cell surface binding analogs of GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha.

22. The method of claim 14 wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 antibodies and fragments thereof, CD28 antibodies and fragments thereof, and combinations of said antibodies and fragments thereof.

23. The method of claim 22 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 antibodies immobilized on coated beads.

24. The method of claim 3 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

25. The method of claim 24 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

26. The method of claim 25 wherein said cytokine is interleukin-2.

27. The method of claim 24 wherein said culturing is for about seven days.

28. The method of claim 24 wherein said culturing is for about 14 days.

29. The method of claim 3 wherein said contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

30. The method of claim 1 wherein the lentiviral vector is present at an MOI of less than 500.

31. A method to introduce genetic material into a living subject comprising introduction of a cell transduced by the method of claim 1.

32. The method of claim 4 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

33. The method of claim 1 wherein said contacting occurs ex vivo.

L1 ANSWER 4 OF 6 USPATFULL on STN
2004:44602 Conditionally replicating vectors for inhibiting viral infections.
Humeau, Laurent, Gaithersburg, MD, UNITED STATES
Li, Yuexia, Gaithersburg, MD, UNITED STATES

Dropulic, Boro, Ellicott City, MD, UNITED STATES
Schonely, Kathy L., Germantown, MD, UNITED STATES
US 2004033595 A1 20040219
APPLICATION: US 2003-388667 A1 20030314 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides improved conditionally replicating vectors that have improved safety against the generation of replication competent vectors or virus. Also disclosed are methods of making, propagating and selectively packaging, modifying, and using such vectors. Included are improved helper constructs, host cells, for use with the improved vectors as well as pharmaceutical compositions and host cells comprising the vectors, the use of vector containing host cells to screen drugs, and methods of using the vectors to determine gene function. The methods also include the prophylactic and therapeutic treatment of disease, especially viral infection, and HIV infection in particular.

CLM What is claimed is:

1. A method of preventing or inhibiting the production of viral DNA in a cell, said method comprising introducing into said cell, before said cell is infected with a virus that produces viral DNA as part of the virus's life cycle, a conditionally replicating viral vector that integrates into said cell's genome, wherein said viral vector does not contain a genetic antiviral agent payload that inhibits said virus upon expression.

2. The method of claim 1 wherein said virus is a wildtype virus.

3. The method of claim 2 wherein said virus is HIV-1.

4. The method of claim 3 wherein said cell is a CD4+ cell.

5. The method of claim 1 wherein said cell is a hematopoietic stem cell.

6. The method of claim 1 wherein said cell is a CD4+ cell.

7. The method of claim 1 wherein said vector integrates into the cell's genome by the same process as that used by said virus.

8. The method of claim 1 wherein said conditionally replicating viral vector is introduced at a multiplicity of infection from about 2 to about 50.

9. The method of claim 1 wherein said cell is a primary cell.

10. The method of claim 9 further comprising introducing said cell into a subject.

11. The method of claim 10 wherein said subject is human.

12. The method of claim 11 further comprising the administration of an antiviral agent to said subject.

13. The method of claim 9 wherein said cell is a human cell.

14. The method of claim 1 wherein said conditionally replicating viral vector is derived from an HIV virus.

15. The method of claim 14 wherein said HIV virus is HIV-1.

16. The method of claim 1 wherein said conditionally replicating vector is a chimeric vector.

17. The method of claim 16 wherein said conditionally replicating vector comprises HIV-1 and HIV-2 elements.

18. The method of claim 1 wherein said conditionally replicating vector is pseudotyped before introducing said vector into said cell.

19. The method of claim 18 wherein said conditionally replicating vector is pseudotyped with the vesicular stomatis virus envelope protein VSV-G, the RD114 envelope protein, the Rabies Virus envelope protein, the Gibbon Ape Leukemia Virus envelope protein, or a chimeric envelope protein.

20. The method of claim 5 wherein said conditionally replicating vector expresses a variant of the O6-methylguanine-DNA-methyltransferase (MGMT) that is resistant to O6-benzylguanine (BG) mediated inactivation and thus protects said cells against alkylating agents.

vectors.

Rumeau, Laurent, Gaithersburg, MD, United States
Han, Wei, Montgomery Village, MD, United States
Lu, Xiaobin, Gaithersburg, MD, United States
Slepushkin, Vladimir, Damascus, MD, United States
Lesher, Mechelle, Frederick, MD, United States
Davis, Brian, Gaithersburg, MD, United States
Chang, Yung-Nien, Cockeysville, MD, United States
Dropulic, Boro, Ellicott City, MD, United States
VIRxSYS Corporation, Gaithersburg, MD, United States (U.S. corporation)

US 6627442 B1 20030930
APPLICATION: US 2000-653088 20000831 (9)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods, as well as compositions related thereto, for the efficient transduction of cells using viral vectors. The efficiency of transduction is increased by contacting the cell to be transduced with one or more molecules that bind the cell surface. Contact with a cell surface binding molecule may occur before, after, or simultaneously with contact between the viral vector and the cell. The transduced vectors may be constructed to express a gene of interest, permitting the transduced cells to be used as therapeutic and prophylactic agents.

CLM What is claimed is:

1. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells comprising contacting the surface of said cells with both an HIV derived lentiviral vector and at least one molecule which binds said cell surface wherein said contacting occurs in vitro or ex vivo and wherein greater than about 90% of the cells are stably transduced after about 14 days.

2. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs before contacting the cells with at least one cell surface binding molecule.

3. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs simultaneously with contacting the cells with at least one cell surface binding molecule.

4. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs after contacting the cells with at least one cell surface binding molecule.

5. The method of claim 1 where said contacting with a lentiviral vector occurs more than once.

6. The method of claim 1 wherein said cell surface binding molecule is an antibody, a ligand or a cell surface molecule.

7. The method of claim 1 wherein said lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, vpr, vpu, tat or rev genes.

8. The method of claim 7 wherein said sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, vpr, vpu, tat or rev genes.

9. The method of claim 1 wherein said lentiviral vector is a pseudotyped vector.

10. The method of claim 9 wherein said pseudotyped vector contains the vesicular stomatitis virus G envelope protein.

11. The method of claim 1 wherein said hematopoietic cell is a CD4 positive cell.

12. The method of claim 1 wherein said hematopoietic cell is a lymphocyte.

13. The method of claim 12 wherein said lymphocyte is a CD4 or CD8 positive cell.

14. The method of claim 1 wherein said hematopoietic cell is a CD34 positive cell.

15. The method of claim 14 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or antibodies that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

16. The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand,

ligand, TPO ligand, or Kit ligand.

17. The method of claim 1 wherein the said cell is a dendritic cell or a cell capable of differentiating into a dendritic cell.

18. The method of claim 17 wherein said at least one cell surface binding molecule is selected from compositions comprising GM-CSF, IL-4, and TNF-alpha; GM-CSF and interferon-alpha; or antibodies that are cell surface binding analogs of GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha.

19. The method of claim 11 wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 antibodies and fragments thereof, CD28 antibodies and fragments thereof, and combinations of said antibodies and fragments thereof.

20. The method of claim 19 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 antibodies immobilized on coated beads.

21. The method of claim 3 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

22. The method of claim 21 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

23. The method of claim 22 wherein said cytokine is interleukin-2.

24. The method of claim 21 wherein said culturing is for about seven days.

25. The method of claim 21 wherein said culturing is for about 14 days.

26. The method of claim 3 wherein said contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

27. The method of claim 1 wherein the lentiviral vector is present at an MOI of less than 500.

28. A method to introduce genetic material into a living subject comprising introduction of a cell transduced by the method of claim 1.

29. The method of claim 4 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

30. The method of claim 1 wherein said contacting occurs ex vivo.

L1 ANSWER 6 OF 6 USPATFULL on STN
2003:37147 Conditionally replicating vectors for inhibiting viral infections.
Humeau, Laurent, Gaithersburg, MD, UNITED STATES
Li, Yuexia, Gaithersburg, MD, UNITED STATES
Merling, Randall, North Potomac, MD, UNITED STATES
Dropulic, Boro, Ellicott City, MD, UNITED STATES
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US 2003026791 A1 20030206
APPLICATION: US 2001-819401 A1 20010327 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides improved conditionally replicating vectors that have improved safety against the generation of replication competent vectors or virus. Also disclosed are methods of making, propagating and selectively packaging, modifying, and using such vectors. Included are improved helper constructs, host cells, for use with the improved vectors as well as pharmaceutical compositions and host cells comprising the vectors, the use of vector containing host cells to screen drugs, and methods of using the vectors to determine gene function. The methods also include the prophylactic and therapeutic treatment of disease, especially viral infection, and HIV infection in particular.
CLM What is claimed is:
1. A method of preventing or inhibiting the production of viral DNA in a cell infected with a virus comprising introducing into said cell, before the cell is infected with said virus, a conditionally replicating viral vector wherein said vector prevents or inhibits the production of viral DNA.

2. The method of claim 1 wherein said viral DNA would have been integrated into the cell's genome.

3. The method of claim 1 wherein said virus is a wildtype virus.

4. The method of claim 1 wherein said cell is a CD4+ cell.

5. The method of claim 3 wherein said virus is HIV-1.

6. The method of claim 1 wherein said vector integrates into the cell's genome by the same process as that used by said virus.

7. The method of claim 1 wherein said vector comprises a gene to be expressed and one or more first nucleotide sequences and wherein the vector: (a) replicates in a host cell only upon complementation with a wild-type strain of virus, a helper virus, or a helper vector, each of which is sensitive to the presence of said one or more first nucleotide sequences; (b) is resistant to the presence of said one or more first nucleotide sequences; and (c) contains one or more substitutions, insertions, or deletions that decrease the likelihood of generating a replication competent vector.

```
=> e han wei/in
E1            5     HAN WAN TAEK/IN
E2            1     HAN WANG C/IN
E3           47 --> HAN WEI/IN
E4            1     HAN WEI JER/IN
E5            4     HAN WEI KUO/IN
E6            3     HAN WEIMIN/IN
E7            4     HAN WEIYU/IN
E8            1     HAN WEN C/IN
E9           13     HAN WEN CHING/IN
E10           1     HAN WEN K/IN
E11           2     HAN WEN YAO/IN
E12           4     HAN WENHAI/IN

=> s e3
L2           47 "HAN WEI"/IN

=> s l2 not l1
L3           45 L2 NOT L1

=> s l3 and (retrovir? or lentivir?)
         41371 RETROVIR?
          6306 LENTIVIR?
L4           2 L3 AND (RETROVIR? OR LENTIVIR?)

=> d l4,ti,1-2

L4   ANSWER 1 OF 2  USPATFULL on STN
TI     N-(substituted benzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamides useful
       as hiv integrase inhibitors

L4   ANSWER 2 OF 2  USPATFULL on STN
TI     Methods for production of non-disease causing hemoglobin by ex vivo
       oligonucleotide gene editing of human stem/progenitor cells


=> e lu xiaobin/in
E1            1     LU XIAO LONG/IN
E2            1     LU XIAOAN/IN
E3            4 --> LU XIAOBIN/IN
E4            1     LU XIAOCHENG/IN
E5            1     LU XIAOCHUAN R/IN
E6            1     LU XIAODAN HU/IN
E7            1     LU XIAODE/IN
E8            1     LU XIAODONG/IN
E9            2     LU XIAOHAI/IN
E10           1     LU XIAOJIA J/IN
E11           4     LU XIAOJIANG/IN
E12          40     LU XIAOLIN/IN

=> s e3
L5            4 "LU XIAOBIN"/IN

=> d l5,ti,1-4

L5   ANSWER 1 OF 4  USPATFULL on STN
TI     Regulation of transcription with a cis-acting ribozyme

L5   ANSWER 2 OF 4  USPATFULL on STN
TI     Lentivirus vector-based approaches for generating an immune response to
       HIV in humans

L5   ANSWER 3 OF 4  USPATFULL on STN
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L5 ANSWER 4 OF 4 USPATFULL on STN
TI Methods for stable transduction of cells with hiv-derived viral vectors

=> s l5 not l1
L6 2 L5 NOT L1

=> d l6,cbib,ab,clm,1-2

L6 ANSWER 1 OF 2 USPATFULL on STN
2005:294724 Regulation of transcription with a cis-acting ribozyme.
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Dropulic, Boro, Ellicott City, MD, UNITED STATES
Slepushkin, Vladimir, Damascus, MD, UNITED STATES
Binder, Gwendolyn, Montgomery Village, MD, UNITED STATES
US 2005257277 A1 20051117
APPLICATION: US 2004-847728 A1 20040517 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a recombinant transcription unit capable of producing an RNA transcript of a predetermined size comprising a regulatory sequence operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said regulatory sequence. The transcribed region comprises a region that encodes for a viral sequence, and a non-coding region downstream of the region encoding for said viral sequence, wherein the non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme. Methods of using the recombinant transcription unit, and cells containing vectors comprising the recombinant transcription unit are also disclosed.
CLM What is claimed is:
1. A method of preparing a recombinant transcription unit capable of producing an RNA transcript of a predetermined size comprising: operably linking a regulatory sequence and a nucleotide sequence comprising a transcribed region such that transcription of said transcribed region is controlled by said regulatory sequence, wherein said transcribed region comprises a region that encodes a viral sequence and a non-coding region downstream of said region encoding for said viral sequence, wherein said non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme.

2. The method of claim 1, wherein said non-coding region further comprises a nucleotide sequence encoding a cleavage signal upstream of said nucleotide sequence encoding a cis-acting ribozyme.

3. The method of claim 2, wherein said cleavage signal is a polyadenylation signal, a transient pause site, a strong pause site, a termination site, a near upstream (NUE), or a 3' untranslated sequence.

4. The method of claim 3, wherein said polyadenylation signal is a bovine growth hormone polyadenylation (poly-A) signal or a S4V0 poly-A site.

5. The method of claim 3, wherein more than one cleavage signal is used.

6. The method of claim 1, wherein said regulatory sequence is a prokaryotic regulatory sequence.

7. The method of claim 1, wherein said regulatory sequence is a eukaryotic regulatory sequence.

8. The method of claim 7, wherein said regulatory sequence is a cytomegalovirus (CMV) promoter or an elongation factor (EF) promoter.

9. The method of claim 1, wherein said viral sequence encodes a viral protein.

10. The method of claim 9, wherein said viral protein is a protein encoded by a lentivirus or a viral envelope protein.

11. The method of claim 9, wherein said viral protein is VSV-G, gag, pol, tat, or rev, or any combination of VSV-G, gag, pol, tat, and rev.

12. The method of claim 9, wherein said viral sequence further comprises a nucleotide sequence encoding an antiviral agent that is either upstream or downstream of the nucleotide sequence encoding said viral protein.

13. The method of claim 12, wherein said antiviral agent is an antisense molecule or a ribozyme.

from satellite or viroid RNA.

15. The method of claim 14, wherein said cis-acting ribozyme is derived from satellite RNA of Tobacco Ringspot Virus or derived from satellite RNA of Arabis mosaic virus.

16. A host cell comprising a recombinant transcription unit capable of producing an RNA transcript of a predetermined size, wherein said transcription unit comprises a regulatory sequence operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said regulatory sequence, wherein said transcribed region comprises a region that encodes for a viral sequence, and a non-coding region downstream of said region encoding for said viral sequence, wherein said non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme.

17. The host cell of claim 16, wherein said non-coding region further comprises a nucleotide sequence encoding a cleavage signal upstream of said nucleotide sequence encoding said cis-acting ribozyme.

18. A recombinant transcription unit capable of producing an RNA transcript of a predetermined size comprising a regulatory sequence operably linked to a nucleotide sequence comprising a transcribed region encoding a viral sequence and a non-coding region downstream of said region encoding for said viral sequence, wherein said non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme.

19. The recombinant transcription unit of claim 18, wherein said non-coding region further comprises a nucleotide sequence encoding a termination cleavage signal upstream of said nucleotide sequence encoding said cis-acting ribozyme.

20. The recombinant transcription unit of claim 19, wherein said cleavage signal is a polyadenylation signal, a pause site, a strong pause site, a near upstream (NUE), or a 3' untranslated sequence.

21. The recombinant transcription unit of claim 20, wherein said polyadenylation signal is a bovine growth hormone polyadenylation (poly-A) signal, or a SV40 poly-A site.

22. The recombinant transcription unit of claim 20, wherein more than one signal is used.

23. The recombinant transcription unit of claim 18, wherein said regulatory sequence is a prokaryotic regulatory sequence.

24. The recombinant transcription unit of claim 18, wherein said regulatory sequence is a eukaryotic regulatory sequence.

25. The recombinant transcription unit of claim 24, wherein said regulatory sequence is a cytomegalovirus (CMV) promoter or an elongation factor (EF) promoter.

26. The recombinant transcription unit of claim 18, wherein said viral sequence is a viral protein.

27. The recombinant transcription unit of claim 26, wherein said viral protein is a protein encoded by a lentivirus or a viral envelope protein.

28. The recombinant transcription unit of claim 26, wherein said viral protein is VSV-G, gag, pol, tat, or rev, or any combination of VSV-G, gag, pol, tat, and rev.

29. The recombinant transcription unit of claim 28, wherein in addition to a nucleotide sequence encoding a viral protein said viral sequence further comprises a nucleotide sequence encoding an antiviral agent that is either upstream or downstream of the nucleotide sequence encoding said viral protein.

30. The recombinant transcription unit of claim 29, wherein said antiviral agent is an antisense molecule or a ribozyme.

31. The recombinant transcription unit of claim 18, wherein said cis-acting ribozyme is derived from satellite or viroid RNA.

32. The recombinant transcription unit of claim 31, wherein said cis-acting ribozyme is derived from satellite RNA of Tobacco Ringspot Virus or derived from satellite RNA of Arabis mosaic virus.

33. A method of limiting the size of an RNA transcript produced from a transcription unit, said method comprising: inducing transcription of a

nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said regulatory sequence, wherein said transcribed region comprises a region that encodes for a viral sequence, and a non-coding region downstream of said region encoding for said viral sequence, wherein said non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme; and wherein said transcription unit produces a transcript under conditions wherein the sequence encoding said cis-acting ribozyme is transcribed and cleaves said transcript in cis.

34. The method of claim 33, wherein said non-coding region further comprises a nucleotide sequence encoding a cleavage signal upstream of said nucleotide sequence encoding a cis-acting ribozyme.

35. The method of claim 33, wherein said cleavage signal is a polyadenylation signal, a transient pause site, a strong pause site, a termination site, a near upstream (NUE), or a 3' untranslated sequence.

36. The method of claim 33, wherein said polyadenylation signal is a bovine growth hormone polyadenylation (poly-A) signal, or a S4V0 poly-A site.

37. The method of claim 35, wherein more than one signal is used.

38. The method of claim 33, wherein said regulatory sequence is a prokaryotic regulatory sequence.

39. The method of claim 33, wherein said regulatory sequence is a eukaryotic regulatory sequence.

40. The method of claim 39, wherein said regulatory sequence is a cytomegalovirus (CMV) promoter or an elongation factor (EF) promoter.

41. The method of claim 33, wherein said viral sequence encodes a viral protein.

42. The method of claim 41, wherein said viral protein is a protein encoded by a lentivirus or a viral envelope protein.

43. The method of claim 41, wherein said viral protein is VSV-G, gag, pol, tat, or rev, or any combination of VSV-G, gag, pol, tat, and rev.

44. The method of claim 41, wherein in addition to a nucleotide sequence encoding a viral protein said viral sequence further comprises a nucleotide sequence encoding an antiviral agent that is either upstream or downstream of the nucleotide sequence encoding said viral protein.

45. The method of claim 44, wherein said antiviral agent is an antisense molecule or a ribozyme.

46. The method of claim 33, wherein said cis-acting ribozyme is derived from satellite or viroid RNA.

47. The method of claim 46, wherein said cis-acting ribozyme is derived from satellite RNA of Tobacco Ringspot Virus or derived from satellite RNA of Arabis mosaic virus.

48. A vector comprising: (a) a first transcription unit capable of producing a first RNA transcript of a predetermined size, wherein said first transcription unit comprises a first promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said first promoter, wherein said transcribed region comprises a region that encodes for a first gene, and a first non-coding region downstream of said region encoding for said first gene, wherein said first non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme; and (b) a second transcription unit capable of producing a second RNA transcript of a predetermined size, wherein said second transcription unit comprises a second promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said second promoter, wherein said transcribed region comprises a region that encodes for a second gene, and a second non-coding region downstream of said region encoding for said second gene, wherein said second non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme.

49. The vector of claim 48, wherein said first and second promoter are different.

50. The vector of claim 48, wherein said first and second promoter non-coding regions comprise a nucleotide sequence encoding a cis-acting ribozyme that is either the same or different.

51. The vector of claim 48 wherein the first gene, second gene, or both have at their carboxy termini a cleavage signal.

52. The vector of claim 51, wherein said cleavage signal is a polyadenylation signal, a transient pause site, a strong pause site, a termination site, a near upstream (NUE), or a 3' untranslated sequence.

53. The vector of claim 52, wherein more than one signal is used.

54. The vector of claim 48, wherein said first cis-acting ribozyme or the second cis-acting ribozyme or both are derived from satellite or viroid RNA.

55. The vector of claim 54, wherein said cis-acting ribozyme is derived from satellite RNA of Tobacco Ringspot Virus or derived from satellite RNA of Arabis mosaic virus.

56. The vector of claim 48, wherein said first promoter is constitutive and said second promoter is inducible.

57. The vector of claim 48, wherein said first gene is different from said second gene.

58. The vector of claim 57, wherein said first gene is a dominant negative transgene and the second gene is a gene that when expressed the expression product can convert the dominant negative transgene into a functional gene.

59. The vector of claim 57, wherein said first gene is a proenzyme and said second gene's expression product converts the proenzyme to an active enzyme.

60. The vector of claim 57, wherein said first gene encodes for a protein in which at least one amino acid of said protein is capable of being phosphorylated and said second gene encodes for a kinase capable of phosphorylating said amino acid of said protein.

61. The vector of claim 57, wherein said first gene encodes for a first protein which comprises at least one phosphorylated amino acid and said second gene encodes for a protein phosphatase capable of dephosphorylating said amino acid of said first protein.

62. A host cell comprising a vector that comprises: (a) a first transcription unit capable of producing a first RNA transcript of a predetermined size, wherein said first transcription unit comprises a first promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said first promoter, wherein said transcribed region comprises a region that encodes for a first gene, and a first non-coding region downstream of said region encoding for said first gene, wherein said first non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme; and (b) a second transcription unit capable of producing a second RNA transcript of a predetermined size, wherein said second transcription unit comprises a second promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said second promoter, wherein said transcribed region comprises a region that encodes for a second gene, and a second non-coding region downstream of said region encoding for said second gene, wherein said second non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme.

63. The host cell of claim 62, wherein the first gene, second gene, or both have at their carboxy termini a cleavage signal.

64. A method of making a transgenic animal comprising inserting into the genome of said animal a vector comprising: (a) a first transcription unit capable of producing a first RNA transcript of a predetermined size, wherein said first transcription unit comprises a first promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said first promoter, wherein said transcribed region comprises a region that encodes for a first gene, and a first non-coding region downstream of said region encoding for said first gene, wherein said first non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme; and (b) a second transcription unit capable of producing a second RNA transcript of a predetermined size, wherein said second transcription unit comprises a second promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said second promoter, wherein said transcribed region comprises a region that encodes for a second gene, and a second non-coding region downstream of

non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme.

65. The method of claim 64, wherein the first gene, second gene, or both have at their carboxy termini a cleavage signal.

66. The method of claim 64, wherein said vector is inserted into the genome of the germline of said animal.

67. The method of claim 64, wherein said vector is inserted into the genome of an unfertilized or fertilized egg of said animal.

68. The method of claim 64, wherein said vector is inserted into the genome of an embryo of said animal.

69. The method of claim 64, wherein said vector is inserted into the genome of a cell located in the uterus of said animal.

70. A transgenic non-human animal comprising a vector which comprises: (a) a first transcription unit capable of producing a first RNA transcript of a predetermined size, wherein said first transcription unit comprises a first promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said first promoter, wherein said transcribed region comprises a region that encodes for a first gene, and a first non-coding region downstream of said region encoding for said first gene, wherein said first non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme; and (b) a second transcription unit capable of producing a second RNA transcript of a predetermined size, wherein said second transcription unit comprises a second promoter operably linked to a nucleotide sequence comprising a transcribed region such that the transcription of said transcribed region is controlled by said second promoter, wherein said transcribed region comprises a region that encodes for a second gene, and a second non-coding region downstream of said region encoding for said second gene, wherein said second non-coding region comprises a nucleotide sequence encoding a cis-acting ribozyme.

71. The transgenic non-human animal of claim 70, wherein the first gene, second gene, or both have at their carboxy termini a cleavage signal.

72. A two vector retrovirus production system comprising: (a) a first vector comprising a nucleotide sequence encoding a payload and a first promoter that controls transcription of said payload; and (b) a second vector comprising: (i) a nucleotide sequence encoding a structural gene and a second promoter which controls transcription of said structural gene; and (ii) a nucleotide sequence encoding a non-structural gene and a third promoter which controls transcription of said non-structural gene, wherein said nucleotide sequence encoding said structural gene and said nucleotide sequence encoding said non-structural gene are separated by a nucleotide sequence encoding a cis-acting ribozyme.

73. The retrovirus production system of claim 72, wherein the first, second, and third promoters are the same or are different.

74. The retrovirus production system of claim 72, wherein the payload is selected from the group consisting of an antisense molecule, a RNA decoy, a transdominant mutant, a toxin, a single-chain antibody (scAb) directed to a viral structural protein, a siRNA, and a ribozyme.

75. The retrovirus production system of claim 72, wherein said structural gene is selected from the group consisting of gag, a gag-pol precursor, pro, reverse transcriptase (RT), integrase (In) and env.

76. The retrovirus production system of claim 72, wherein said non-structural gene is selected from the group consisting of tat, rev, nef, vpr, vpu, and vif.

77. A two vector retrovirus production system comprising: (a) a first vector comprising a nucleotide sequence encoding a payload and a first promoter that controls transcription of said payload; and (b) a second vector comprising (i) a nucleotide sequence encoding a structural gene and a second promoter that controls transcription of said structural gene, (ii) a nucleotide sequence encoding a non-structural gene and a third promoter that controls transcription of said non-structural gene, and (iii) a nucleotide sequence encoding an envelope gene and a fourth promoter that controls transcription of said envelope gene, wherein each of the nucleotide sequences encoding the three genes are separated by a nucleotide sequence encoding a cis-ribozyme.

78. The retrovirus production system of claim 77, wherein the first, second, third, and fourth promoters are the same or are different.

79. The retrovirus production system of claim 77, wherein the payload is selected from the group consisting of an antisense molecule, a RNA decoy, a transdominant mutant, a toxin, a single-chain antibody (scAb) directed to a viral structural protein, a siRNA, and a ribozyme.

80. The retrovirus production system of claim 77, wherein said structural gene is selected from the group consisting of gag, a gag-pol precursor, pro, reverse transcriptase (RT), integrase (In) and env.

81. The retrovirus production system of claim 77, wherein said non-structural gene is selected from the group consisting of tat, rev, nef, vpr, vpu, and vif.

82. A method of producing a retrovirus comprising contacting a cell with a two vector retrovirus production system comprising: (a) a first vector comprising a nucleotide sequence encoding a payload and a first promoter that controls transcription of said payload; and (b) a second vector comprising a nucleotide sequence encoding a structural gene and a second promoter that controls transcription of said structural gene, a nucleotide sequence encoding a non-structural gene and a third promoter that controls transcription of said non-structural gene, wherein said nucleotide sequence encoding said structural gene and said nucleotide sequence encoding said non-structural gene are separated by a nucleotide sequence encoding a cis-acting ribozyme.

83. A method of producing a retrovirus comprising contacting a cell with a two vector retrovirus production system comprising: (a) a first vector comprising a nucleotide sequence encoding a payload and a first promoter that controls transcription of said payload; and (b) a second vector comprising a nucleotide sequence encoding a structural gene and a second promoter that controls transcription of said structural gene, a nucleotide sequence encoding a non-structural gene and a third promoter that controls transcription of said non-structural gene, and a nucleotide sequence encoding an envelope gene and a fourth promoter that controls transcription of said envelope gene, wherein each of the nucleotide sequences encoding the three genes are separated by a nucleotide sequence encoding a cis-ribozyme.

L6 ANSWER 2 OF 2 USPATFULL on STN
2005:226532 Lentivirus vector-based approaches for generating an immune response to HIV in humans.
Lu, Xiaobin, Germantown, MD, UNITED STATES
Dropulic, Boro, Ellicott City, MD, UNITED STATES
US 2005196381 A1 20050908
APPLICATION: US 2004-937026 A1 20040909 (10)
PRIORITY: US 2003-501665P 20030909 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to multiple novel approaches for the generation of an immune response in humans using lentivirus-based vector technology. The invention provides for the ability to mimic the efficacy of a live attenuated (LA) vaccine, without exposing the patient to the risk of disease as possible with some LA vaccines. The invention thus provides for systems of complementary conditionally replicating vectors, vectors that produce replication deficient virus like particles, and multi-antigen constructs that target a virus or microbial pathogen. The use of these materials in the practice of the invention permits the generation of robust cellular and humoral responses to the antigens presented thereby.
CLM What is claimed is:
1. A method of inducing an immune response in a subject, said method comprising administering a system of two or more conditionally replicating lentiviral vectors to a cell of said subject, wherein each of said two or more vectors replicates only in the presence of the other vectors in the system, and said system of vectors expresses one or more antigens to which an immune response is desired in said subject.

2. The method of claim 1 wherein at least one of said vectors contains a genetic antiviral agent against one or more other vectors in said system.

3. The method of claim 1 wherein said system comprises two lentiviral vectors.

4. The method of claim 2 wherein only one of said vectors comprises an env encoding sequence.

5. The method of any one of claims 1-4 wherein said administering occurs ex vivo.

6. The method of any one of claims 1-4 wherein said immune response is a

cells.

7. The method of claim 5 wherein said immune response is a cellular in nature and comprises the potentiation of CTL and/or CD4+ cells.

8. The method of claims 1-4 wherein said immune response is protective against a virus or microorganism expressing one or more antigens expressed by said vectors.

9. The method of claim 5 wherein said immune response is protective against a virus or microorganism expressing one or more antigens expressed by said vectors.

10. The method of any one of claims 1-4 wherein said one or more antigens is one or more HIV antigens.

11. The method of claim 5 wherein said one or more antigens is one or more HIV antigens.

12. A method of inducing an immune response in a subject, said method comprising administering a system of two or more lentiviral vectors to a cell of said subject, wherein said system of vectors expresses the proteins needed to form a virus like-particle, and at least one of said vectors cannot be packaged into said particle.

13. A replication deficient lentiviral vector comprising a deletion of all or part of the central polypurine tract and a heterologous promoter capable of directing expression of viral proteins encoded by said vector.

14. A method of inducing an immune response in a subject, said method comprising administering a replication deficient lentiviral vector according to claim 13 to a cell of said subject, wherein said vector expresses the proteins needed to form a virus like-particle.

15. The method of any one of claims 1-4, 12 and 14, wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

16. The method of claim 5 wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

17. The method of claim 6 wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

18. The method of claim 7 wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

19. The method of claim 8 wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

20. The method of claim 9 wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

21. The method of claim 10 wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

22. The method of claim 11 wherein said one or more antigens is expressed by a multi-antigen encoding construct which results in the expression of multiple viral epitopes as a single polypeptide.

23. The method of any one of claims 1-4, 12 and 14, wherein said vector comprises a sequence encoding a non-lentiviral antigen.

24. The method of claim 5 wherein said vector comprises a sequence encoding a non-lentiviral antigen.

25. The method of claim 6 wherein said vector comprises a sequence encoding a non-lentiviral antigen.

26. The method of claim 7 wherein said vector comprises a sequence encoding a non-lentiviral antigen.

27. The method of claim 8 wherein said vector comprises a sequence encoding a non-lentiviral antigen.

28. The method of claim 9 wherein said vector comprises a sequence encoding a non-lentiviral antigen.

29. The method of claim 10 wherein said vector comprises a sequence encoding a non-lentiviral antigen.

30. The method of claim 11 wherein said vector comprises a sequence encoding a non-lentiviral antigen.

31. The method of any one of claims 1-4, 12, and 14, wherein said vector comprises a sequence encoding a non-lentiviral antigen, and wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

32. The method of claim 24 wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

33. The method of claim 25 wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

34. The method of claim 26 wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

35. The method of claim 27 wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

36. The method of claim 28 wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

37. The method of claim 29 wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

38. The method of claim 30 wherein said non-lentiviral antigen is from a virus selected from the group consisting of retroviruses, togaviruses, rhabdoviruses, paramyxoviruses, herpesviruses, orthomyxoviruses, and coronaviruses.

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=> e slepushkin vladimir/in
E1            2     SLEPTSOV VASILY MIKHAILOVICH/IN
E2            5     SLEPTSOVA IRINA/IN
E3            5 --> SLEPUSHKIN VLADIMIR/IN
E4            3     SLEPYAN DAVID H/IN
E5            1     SLEPYAN EDWARD/IN
E6            2     SLESAK CHRISTIAN H/IN
E7            1     SLESAR MILAN/IN
E8            3     SLESARENKO VLADIMIR F/IN
E9            3     SLESAREV ALEXEI I/IN
E10           1     SLESAREV BORIS A/IN
E11           5     SLESAREV VLADIMIR I/IN
E12           1     SLESAREV VLADIMIR IVANOVICH/IN

=> s e3
L7            5 "SLEPUSHKIN VLADIMIR"/IN

=> s l7 not l1
L8            2 L7 NOT L1

=> d l8,ti,1-2

L8   ANSWER 1 OF 2  USPATFULL on STN
TI     Regulation of transcription with a cis-acting ribozyme

L8   ANSWER 2 OF 2  USPATFULL on STN
TI     Non-ligand polypeptide and liposome complexes as intracellular delivery
       vehicles
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E1            1      LESHER M KENNETH/IN
E2            3      LESHER MARK K/IN
E3            2 --> LESHER MECHELLE/IN
E4            1      LESHER MELVIN L/IN
E5            1      LESHER ROGER P/IN
E6            1      LESHER THOMAS/IN
E7            4      LESHER TIM/IN
E8            9      LESHER TIMOTHY/IN
E9            1      LESHER TIMOTHY E/IN
E10           2      LESHER TOMMY G/IN
E11           1      LESHER VERNON HAYES/IN
E12           1      LESHESKI LAWRENCE E/IN

=> s e3
L9            2 "LESHER MECHELLE"/IN

=> d l9,ti,1-2

L9   ANSWER 1 OF 2  USPATFULL on STN
TI     Methods for stable transduction of cells with viral vectors

L9   ANSWER 2 OF 2  USPATFULL on STN
TI     Methods for stable transduction of cells with hiv-derived viral vectors


=> d l9,cbib,ab,clm,1-2

L9   ANSWER 1 OF 2  USPATFULL on STN
2004:82307 Methods for stable transduction of cells with viral vectors.
    Humeau, Laurent, Germantown, MD, UNITED STATES
    Han, Wei, Montgomery Village, MD, UNITED STATES
    Lu, Xiaobin, Germantown, MD, UNITED STATES
    Slepushkin, Vladimir, Damascus, MD, UNITED STATES
    Lesher, Mechelle, Columbia, MD, UNITED STATES
    Davis, Brian, Gaithersburg, MD, UNITED STATES
    Chang, Yung-Nien, Cockeysville, MD, UNITED STATES
    Dropulic, Boro, Ellicott City, MD, UNITED STATES
    US 2004062756 A1 20040401
    APPLICATION: US 2003-664331  A1  20030916 (10)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention provides methods, as well as compositions related
       thereto, for the efficient transduction of cells using viral vectors.
       The efficiency of transduction is increased by contacting the cell to be
       transduced with one or more molecules that bind the cell surface.
       Contact with a cell surface binding molecule may occur before, after, or
       simultaneously with contact between the viral vector and the cell. The
       transduced vectors may be constructed to express a gene of interest,
       permitting the transduced cells to be used as therapeutic and
       prophylactic agents.
CLM    What is claimed is:
       1. A method for stable transduction of primary cells of the
       hematopoietic system and/or hematopoietic stem cells comprising
       contacting the surface of said cells with both a lentiviral vector and
       at least one molecule which binds said cell surface wherein said
       contacting occurs in vitro or ex vivo and  wherein greater than about
       90% of the cells are stably transduced after about 14 days.

       2. The method of claim 1 wherein said contacting the cells with a
       lentiviral vector occurs before contacting the cells with at least one
       cell surface binding molecule.

       3. The method of claim 1 wherein said contacting the cells with a
       lentiviral vector occurs simultaneously with contacting the cells with
       at least one cell surface binding molecule.

       4. The method of claim 1 wherein said contacting the cells with a
       lentiviral vector occurs after contacting the cells with at least one
       cell surface binding molecule.

       5. The method of claim 1 where said contacting with a lentiviral vector
       occurs more than once.

       6. The method of claim 1 wherein said lentiviral vector is derived from
       HIV-1.

       7. The method of claim 1 wherein said cell surface binding molecule is
       an antibody, a ligand or a cell surface molecule.

       8. The method of claim 1 wherein said lentiviral vector comprises at
       least one cis-acting nucleotide sequence derived from the gag, pol, env,
       vif, vpr, vpu, tat or rev genes.
```

9. The method of claim 8 wherein said sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, vpr, vpu, tat or rev genes.

10. The method of claim 1 wherein said lentiviral vector is derived from HIV-2.

11. The method of claim 1 wherein said lentiviral vector is a pseudotyped vector.

12. The method of claim 11 wherein said pseudotyped vector contains the vesicular stomatitis virus G envelope protein.

13. The method of claim 1 wherein said lentiviral vector is a chimeric vector comprising HIV-1 and HIV-2 sequences.

14. The method of claim 1 wherein said hematopoietic cell is a CD4 positive cell.

15. The method of claim 1 wherein said hematopoietic cell is a lymphocyte.

16. The method of claim 15 wherein said lymphocyte is a CD4 or CD8 positive cell.

17. The method of claim 1 wherein said hematopoietic cell is a CD34 positive cell.

18. The method of claim 17 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or antibodies that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

19. The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or antibodies that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

20. The method of claim 1 wherein the said cell is a dendritic cell or a cell capable of differentiating into a dendritic cell.

21. The method of claim 20 wherein said at least one cell surface binding molecule is selected from compositions comprising GM-CSF, IL-4, and TNF-alpha; GM-CSF and interferon-alpha; or antibodies that are cell surface binding analogs of GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha.

22. The method of claim 14 wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 antibodies and fragments thereof, CD28 antibodies and fragments thereof, and combinations of said antibodies and fragments thereof.

23. The method of claim 22 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 antibodies immobilized on coated beads.

24. The method of claim 3 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

25. The method of claim 24 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

26. The method of claim 25 wherein said cytokine is interleukin-2.

27. The method of claim 24 wherein said culturing is for about seven days.

28. The method of claim 24 wherein said culturing is for about 14 days.

29. The method of claim 3 wherein said contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

30. The method of claim 1 wherein the lentiviral vector is present at an MOI of less than 500.

31. A method to introduce genetic material into a living subject comprising introduction of a cell transduced by the method of claim 1.

32. The method of claim 4 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

L9 ANSWER 2 OF 2 USPATFULL on STN
2003:260677 Methods for stable transduction of cells with hiv-derived viral vectors.
Humeau, Laurent, Gaithersburg, MD, United States
Han, Wei, Montgomery Village, MD, United States
Lu, Xiaobin, Gaithersburg, MD, United States
Slepushkin, Vladimir, Damascus, MD, United States
Lesher, Mechelle, Frederick, MD, United States
Davis, Brian, Gaithersburg, MD, United States
Chang, Yung-Nien, Cockeysville, MD, United States
Dropulic, Boro, Ellicott City, MD, United States
VIRxSYS Corporation, Gaithersburg, MD, United States (U.S. corporation)
US 6627442 B1 20030930
APPLICATION: US 2000-653088 20000831 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods, as well as compositions related thereto, for the efficient transduction of cells using viral vectors. The efficiency of transduction is increased by contacting the cell to be transduced with one or more molecules that bind the cell surface. Contact with a cell surface binding molecule may occur before, after, or simultaneously with contact between the viral vector and the cell. The transduced vectors may be constructed to express a gene of interest, permitting the transduced cells to be used as therapeutic and prophylactic agents.
CLM What is claimed is:
1. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells comprising contacting the surface of said cells with both an HIV derived lentiviral vector and at least one molecule which binds said cell surface wherein said contacting occurs in vitro or ex vivo and wherein greater than about 90% of the cells are stably transduced after about 14 days.

2. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs before contacting the cells with at least one cell surface binding molecule.

3. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs simultaneously with contacting the cells with at least one cell surface binding molecule.

4. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs after contacting the cells with at least one cell surface binding molecule.

5. The method of claim 1 where said contacting with a lentiviral vector occurs more than once.

6. The method of claim 1 wherein said cell surface binding molecule is an antibody, a ligand or a cell surface molecule.

7. The method of claim 1 wherein said lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, vpr, vpu, tat or rev genes.

8. The method of claim 7 wherein said sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, vpr, vpu, tat or rev genes.

9. The method of claim 1 wherein said lentiviral vector is a pseudotyped vector.

10. The method of claim 9 wherein said pseudotyped vector contains the vesicular stomatitis virus G envelope protein.

11. The method of claim 1 wherein said hematopoietic cell is a CD4 positive cell.

12. The method of claim 1 wherein said hematopoietic cell is a lymphocyte.

13. The method of claim 12 wherein said lymphocyte is a CD4 or CD8 positive cell.

14. The method of claim 1 wherein said hematopoietic cell is a CD34 positive cell.

15. The method of claim 14 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or antibodies that are cell surface binding analogs

16. The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or antibodies that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

17. The method of claim 1 wherein the said cell is a dendritic cell or a cell capable of differentiating into a dendritic cell.

18. The method of claim 17 wherein said at least one cell surface binding molecule is selected from compositions comprising GM-CSF, IL-4, and TNF-alpha; GM-CSF and interferon-alpha; or antibodies that are cell surface binding analogs of GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha.

19. The method of claim 11 wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 antibodies and fragments thereof, CD28 antibodies and fragments thereof, and combinations of said antibodies and fragments thereof.

20. The method of claim 19 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 antibodies immobilized on coated beads.

21. The method of claim 3 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

22. The method of claim 21 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

23. The method of claim 22 wherein said cytokine is interleukin-2.

24. The method of claim 21 wherein said culturing is for about seven days.

25. The method of claim 21 wherein said culturing is for about 14 days.

26. The method of claim 3 wherein said contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

27. The method of claim 1 wherein the lentiviral vector is present at an MOI of less than 500.

28. A method to introduce genetic material into a living subject comprising introduction of a cell transduced by the method of claim 1.

29. The method of claim 4 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

30. The method of claim 1 wherein said contacting occurs ex vivo.

```
=> e davis brian/in
E1            1     DAVIS BRETT ALLEN/IN
E2            2     DAVIS BRETT L/IN
E3           14 --> DAVIS BRIAN/IN
E4            2     DAVIS BRIAN C/IN
E5            2     DAVIS BRIAN E/IN
E6            3     DAVIS BRIAN F/IN
E7            1     DAVIS BRIAN FREDERICK/IN
E8            3     DAVIS BRIAN J/IN
E9            8     DAVIS BRIAN K/IN
E10           1     DAVIS BRIAN KEITH/IN
E11           1     DAVIS BRIAN KEVIN/IN
E12           6     DAVIS BRIAN L/IN

=> s e3
L10          14 "DAVIS BRIAN"/IN

=> d his

     (FILE 'HOME' ENTERED AT 23:13:49 ON 19 MAR 2006)

     FILE 'USPATFULL' ENTERED AT 23:14:08 ON 19 MAR 2006
                E HUMEAU LAURENT/IN
L1            6 S E3
                E HAN WEI/IN
L2           47 S E3
L3           45 S L2 NOT L1
L4            2 S L3 AND (RETROVIR? OR LENTIVIR?)
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L5             4 S E3
L6             2 S L5 NOT L1
                 E SLEPUSHKIN VLADIMIR/IN
L7             5 S E3
L8             2 S L7 NOT L1
                 E LESHER MECHELLE/IN
L9             2 S E3
                 E DAVIS BRIAN/IN
L10           14 S E3

=> s l10 not (l1 or l2 or l5 or l7 or l9)
L11           11 L10 NOT (L1 OR L2 OR L5 OR L7 OR L9)

=> s l11 and (retrovir? or lentivir?)
          41371 RETROVIR?
           6306 LENTIVIR?
L12           0 L11 AND (RETROVIR? OR LENTIVIR?)

=> e dropulic boro/in
E1             1     DROPSY PATRICK J/IN
E2             3     DROPSY PHILIPPE/IN
E3            19 --> DROPULIC BORO/IN
E4             1     DROR AMIT BEN/IN
E5             1     DROR ASAEL/IN
E6             4     DROR GIDEON/IN
E7             1     DROR GIDON/IN
E8            10     DROR ITAI/IN
E9             4     DROR JACOB/IN
E10            2     DROR LEON L/IN
E11            1     DROR MEIR/IN
E12            1     DROR MENASHE/IN

=> s e3
L13           19 "DROPULIC BORO"/IN

=> s l13 not (l1 or l2 or l5 or l7 or l9)
L14           12 L13 NOT (L1 OR L2 OR L5 OR L7 OR L9)

=> s l14 and (retrovir? or lentivir?)
          41371 RETROVIR?
           6306 LENTIVIR?
L15           12 L14 AND (RETROVIR? OR LENTIVIR?)

=> d l15,ti,1-12

L15  ANSWER 1 OF 12  USPATFULL on STN
TI     Conditionally replicating viral vectors and their use

L15  ANSWER 2 OF 12  USPATFULL on STN
TI     Methods and compositions for identifying gene function

L15  ANSWER 3 OF 12  USPATFULL on STN
TI     Conditionally replicating viral vectors and their use

L15  ANSWER 4 OF 12  USPATFULL on STN
TI     Regulated nucleic acid expression system

L15  ANSWER 5 OF 12  USPATFULL on STN
TI     Lentiviral vectors

L15  ANSWER 6 OF 12  USPATFULL on STN
TI     Methods to assay gene function with viral vectors

L15  ANSWER 7 OF 12  USPATFULL on STN
TI     Methods to inhibit replication of infective virus

L15  ANSWER 8 OF 12  USPATFULL on STN
TI     Conditionally replicating viral vectors and their use

L15  ANSWER 9 OF 12  USPATFULL on STN
TI     Genetic antiviral agents and methods for their use

L15  ANSWER 10 OF 12  USPATFULL on STN
TI     Methods to express genes from viral vectors

L15  ANSWER 11 OF 12  USPATFULL on STN
TI     Method of using a conditionally replicating viral vector to express a
       gene

L15  ANSWER 12 OF 12  USPATFULL on STN
TI     Methods to prepare conditionally replicating viral vectors
```

=> d l15,cbib,ab,clm,5

L15 ANSWER 5 OF 12 USPATFULL on STN
2002:340252 **Lentiviral** vectors.
Dropulic, Boro, Ellicott City, MD, United States
Pitha-Rowe, Paula, Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 6498033 B1 20021224
APPLICATION: US 2000-524004 20000313 (9)
PRIORITY: US 1993-32800P 19931128 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.
CLM What is claimed is:
1. A **retroviral** vector comprising 5' and 3' long terminal repeats (LTRs) derived from a **Lentivirus**, a truncated gag gene sequence containing the **Lentiviral** packaging signal, and a heterologous nucleic acid sequence operably linked 3' of the truncated gag gene sequence to permit its expression by the 5' LTR.

2. The vector of claim 1 wherein said truncated gag gene lacks sequences 3' of the Pst I site within said gag gene and said gag gene is that of pNL4-3.

3. The vector of claim 1 wherein said vector lacks **Lentiviral** sequences from the truncated gag gene to within the nef gene.

4. The vector of claim 1 wherein said **Lentivirus** is HIV-1 or HIV-2.

5. The vector of claim 4 wherein said **Lentivirus** is HIV-1.

6. The vector of claim 1 wherein said heterologous nucleic acid sequence is or encodes a genetic antiviral agent.

7. The vector of claim 1 wherein said genetic antiviral agent is selected from the group consisting of antisense molecules, RNA decoys, transdominant mutants, toxins, single-chain antibodies (scAbs) directed to a viral structural protein, and ribozymes.

8. The vector of claim 7 wherein said genetic antiviral agent is a ribozymne.

9. The vector of claim 1 further comprising a promoter 3' of said heterologous nucleic acid sequence.

10. The vector of claim 1 further comprising an inserted rev responsive element (RRE).

=> d l15,cbib,ab,clm,1-12

L15 ANSWER 1 OF 12 USPATFULL on STN
2004:286242 Conditionally replicating viral vectors and their use.
Dropulic, Boro, Ellicott City, MD, UNITED STATES
Pitha, Paula M., Baltimore, MD, UNITED STATES
US 2004224404 A1 20041111
APPLICATION: US 2002-328643 A1 20021223 (10)
PRIORITY: US 1995-32800P 19951128 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy

CLM What is claimed is:

1. (Cancelled)

2. (Cancelled)

3. (Cancelled)

4. (Cancelled)

5. (Cancelled)

6. (Cancelled)

7. (Cancelled)

8. (Cancelled)

9. (Cancelled)

10. (Cancelled)

11. (Cancelled)

12. (Cancelled)

13. (Cancelled)

14. (Cancelled)

15. (Cancelled)

16. (Cancelled)

17. (Cancelled)

18. (Cancelled)

19. (Cancelled)

20. (Cancelled)

21. (Cancelled)

22. (Cancelled)

23. (Cancelled)

24. (Cancelled)

25. (Cancelled)

26. (Cancelled)

27. (Cancelled)

28. (Cancelled)

29. A method of stimulating an immune response in a human subject, wherein said method comprises contacting a cell of said subject with a conditionally replicating human immunodeficiency viral (crHIV) vector comprising a first nucleotide sequence, wherein said contacting occurs ex vivo, and stimulating an immune response in said subject upon conditional replication of said crHIV after infection of said cell by wild-type HIV virus or after introduction of a helper vector, wherein said first sequence adversely affects said HIV; and wherein said crHIV expresses one or more HIV proteins necessary for replication of said crHIV vector or packaging said crHIV into a virion; and wherein said crHIV viral vector replicates in a host cell only upon complementation with a wild-type virus or a helper virus, or a helper vector, and wherein said complementation renders the host cell permissive for replication of said crHIV vector; and wherein said crHIV vector is selectively replicated over said wild-type virus, helper virus, or helper vector.

30. The method of claim 29 wherein said conditional replication of crHIV results in a persistent expression of said one or more HIV proteins necessary for replication of said crHIV vector.

31. The method of claim 29 wherein said conditional replication of crHIV results in a persistent expression of said one or more HIV proteins necessary for packaging of said crHIV vector into a virion.

comprises or encodes, in which case it also expresses, a genetic antiviral agent.

33. The method of claim 32 wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, an antigen, a receptor, or a suicide gene.

34. The method of claim 33 wherein said genetic antiviral agent is a cytokine, an antigen, or a suicide gene.

35. The method of claim 29 wherein said first nucleotide sequence encodes a mutated HIV protein.

36. The method of claim 29 wherein said first nucleotide sequence is operably linked to the LTR of said crHIV vector.

37. The method of claim 29 wherein said cell of a subject is infected with HIV before said contacting with a crHIV vector.

38. The method of claim 29 wherein said cell of a subject is not infected with HIV before said contacting with a crHIV vector.

39. The method of claim 28 further comprising introducing a helper vector into said cell.

40. The method of claim 29 wherein said helper vector is replicated in said cell and/or packaged into a virion via complementation by said crHIV vector.

41. The method of claim 40 wherein said helper vector comprises a nucleotide sequence that reduces recombination with said crHIV vector.

42. The method of claim 41 wherein said nucleotide sequence that reduces recombination with said crHIV vector is a ribozyme.

43. The method of claim 29 wherein said cell of a subject is an antigen presenting cell or a T cell.

44. The method of claim 43 wherein said cell of a subject is an antigen presenting cell.

45. The method of claim 43 wherein said cell of a subject is a T cell.

46. The method of claim 29 wherein said crHIV vector is packaged in an infectious viral particle or formulated in a liposome or with an adjuvant.

47. The method of claim 29 wherein said crHIV vector is a chimeric vector comprising sequences derived from different viruses.

48. A pair of vectors, said pair comprising a conditionally replicating human immunodeficiency viral (crHIV) vector comprising a first nucleotide sequence and a helper vector which complements replication of said crHIV vector, wherein said first sequence adversely affects wild-type HIV virus and said helper vector; and wherein said crHIV viral vector replicates in a host cell only upon complementation with a wild-type virus or a helper virus, or a helper vector, and wherein said complementation renders the host cell permissive for replication of said crHIV vector; and wherein said crHIV vector is selectively replicated over said wild-type virus, helper virus, or helper vector.

49. The pair of claim 48 wherein said helper vector comprises a nucleotide sequence that reduces recombination with said crHIV vector.

50. The pair of claim 49 wherein said nucleotide sequence that reduces recombination with said crHIV vector is a ribozyme.

51. The pair of claim 48 wherein said first nucleotide sequence comprises or encodes, in which case it also expresses, a genetic antiviral agent.

52. The pair of claim 51 wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, an antigen, a receptor, or a suicide gene.

53. The pair of claim 48 wherein said first nucleotide sequence encodes a mutated HIV protein.

54. The pair of claim 48 wherein one or both of said vectors are packaged in an infectious viral partial or formulated in a liposome or

55. The pair of claim 48 wherein said crHIV vector is a chimeric vector comprising sequences derived from different viruses.

L15 ANSWER 2 OF 12 USPATFULL on STN
2004:260520 Methods and compositions for identifying gene function.
Dropulic, Boro, Ellicott City, MD, UNITED STATES
US 2004203017 A1 20041014
APPLICATION: US 2003-627940 A1 20030725 (10)
PRIORITY: US 2001-264272P 20010125 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods and compositions for the efficient identification of one or more functionalities of a product encoded by a nucleic acid sequence of interest. The methods utilize the abilities to over and/or under express the product in a cell, as well as the combination of these results, to permit the identification of at least one of the product's cellular or in vivo functionality.

CLM What is claimed is:

1. A method of identifying a function of a gene sequence of interest in a cell type comprising a) over expressing all or part of said sequence in a first population of said cell type; b) inhibiting expression of said sequence in a second population of said cell type; c) detecting changes in one or more cellular factors in said first and second populations; d) identifying a function of said gene sequence of interest based on the identity of, or effect on, said one or more cellular factors.

2. The method of claim 1 wherein said changes are increases and/or decreases in the expression of said cellular factors.

3. The method of claim 1 wherein said changes are in the post-translational modifications of said cellular factors.

4. The method of claim 3 wherein said changes are in the phosphorylation or glycosylation of said cellular factors.

5. The method of claim 1 wherein said changes are in the activity of said cellular factors.

6. The method of claim 1 wherein said over expressing in a first population is by use of a pseudotyped **lentiviral** vector.

7. The method of claim 1 wherein said inhibiting expression in a second population is by use of a pseudotyped **lentiviral** vector capable of expressing all or part of said gene sequence in an antisense orientation.

8. The method of claim 1 wherein said inhibiting expression in a second population is by use of a pseudotyped **lentiviral** vector capable of expressing one or more ribozymes against said gene sequence.

9. The method of claim 1 wherein said inhibiting expression in a second population is by the generation of post-transcriptional gene silencing (PTGS) against said gene sequence.

10. The method of claim 1 wherein said cell type is a primary cell.

11. The method of claim 1 wherein said cell type is a cultured cell line.

12. The method of claim 1 wherein said gene sequence of interest was previously identified as expressed in cells of said cell type.

13. The method of claim 1 wherein said gene sequence of interest was not previously identified as expressed in cells of said cell type.

14. The method of claim 1 wherein said gene sequence of interest encodes a product which modulates expression of said one or more cellular factors by binding to nucleic acids encoding, or regulating the expression of, said one or more cellular factors.

15. The method of claim 12 wherein said gene sequence of interest encodes a transcriptional activator.

16. The method of claim 12 wherein said gene sequence of interest encodes a transcriptional repressor.

17. The method of claim 1 wherein said gene sequence of interest is a human sequence.

19. A method of altering the expression of one or more cellular factors in a cell comprising over expressing or inhibiting the expression of a gene sequence for which a function was identified by the method of claim 1.

20. A method of altering the phenotype of a cell comprising over expressing or inhibiting the expression of a gene sequence for which a function was identified by the method of claim 1.

21. A method of identifying a function of a gene sequence of interest in a cell heterologous to the cellular source of said sequence comprising a) over expressing all or part of said sequence in a first population of said cell type; b) inhibiting expression of said sequence in a second population of said cell type; c) detecting changes in one or more cellular factors in said first and second populations; d) identifying said function of said gene sequence of interest based on the identity of, or effect on, said one or more cellular factors.

L15 ANSWER 3 OF 12 USPATFULL on STN
2004:260502 Conditionally replicating viral vectors and their use.
Dropulic, Boro, Ellicott City, MD, UNITED STATES
Pitha, Paula M., Baltimore, MD, UNITED STATES
US 2004202999 A1 20041014
APPLICATION: US 2004-841291 A1 20040507 (10)
PRIORITY: US 1995-32800P 19951128 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.

CLM What is claimed:

1. A **retroviral** vector comprising: (a) 5' and 3' long terminal repeats (LTRs) derived from a **lentivirus**, wherein the 5' LTR is modified to comprise sequences that are not derived from said **lentivirus** whereby control of expression by said modified 5' LTR differs from control of expression by an unmodified LTR; and (b) a heterologous nucleic acid sequence operably linked 3' of the 5' LTR to permit control of its expression by the 5' LTR; and wherein said 3' LTR is downstream of the heterologous sequence.

2. The **retroviral** vector of claim 1, wherein the **lentivirus** is human immunodeficiency virus-1 (HIV-1) or human immunodeficiency virus-2 (HIV-2).

3. The **retroviral** vector of claim 1, wherein the vector conditionally replicates.

4. The **retroviral** vector of claim 3, wherein the vector is a conditionally replicating human immunodeficiency virus (crHIV) vector.

5. The vector of claim 1, wherein the 5' LTR region comprises a promoter and/or enhancer that is not derived from said **lentivirus**.

6. The vector of claim 5, wherein the enhancer is a silencer.

7. The vector of claim 5, wherein the promoter and/or enhancer responds to a cytokine.

8. The vector of claim 7, wherein the response comprises an increase in transcription from the promoter.

9. The vector of claim 7, wherein the response comprises a decrease in transcription from the promoter.

10. The vector of claim 7, wherein the cytokine is selected from the group consisting of interleukins, lymphokines, monokines, interferons, colony stimulating factors, and chemokines.

11. The vector of claim 10, wherein the cytokine is selected from the group consisting of IL-2, tumor necrosis factor α, and RANTES.

12. The vector of claim 5, wherein the promoter and/or enhancer is

13. The vector of claim 5, wherein the promoter and/or enhancer is tumor-specific.

14. The vector of claim 5, wherein the promoter responds to a DNA binding protein.

15. The vector of claim 1, wherein the heterologous nucleic acid sequence is a sequence transcribed to an RNA selected from the group consisting of an antisense molecule, an RNA decoy, and a ribozyme, or the heterologous nucleic acid sequence encodes a protein selected from the group consisting of a transdominant mutant, a toxin, and a single-chain antibody (scAb) directed to a viral structural protein.

16. A host cell comprising the vector of any of claims 1-15.

17. A host cell comprising the vector of claim 7, wherein said cell further comprises a cytokine that is native to the cell.

18. A host cell comprising the vector of claim 7, wherein said cell further comprises a cytokine that is provided to the cell.

19. The host cell of claim 18, wherein the cytokine is provided to the cell by a second vector.

20. A method for cell specific replication and/or propagation of a **retroviral** vector, said method comprising culturing the host cell of claim 16.

21. A method to express a heterologous nucleic acid sequence in a specific cell comprising culturing the cells of claim 16.

22. A method to express a heterologous nucleic acid sequence in a specific cell comprising culturing the cells of claim 17.

23. A method to express a heterologous nucleic acid sequence in a specific cell comprising culturing the cells of claim 18.

24. A method to express a heterologous nucleic acid sequence in a specific cell comprising culturing the cells of claim 19.

25. The vector of claim 1, which lacks a nucleotide sequence encoding Tat.

26. A recombinant host cell which contains the vector of claim 25.

27. A method to express a heterologous nucleic acid sequence in a specific cell comprising contacting the cell of claim 26 with a helper vector comprising a nucleotide sequence encoding Tat, operably linked to a promoter for its expression.

28. The method of claim 27, wherein the **lentivirus** is human immunodeficiency virus-1 (HIV-1) or human immunodeficiency virus-2 (HIV-2).

29. The vector of claim 25, wherein the vector conditionally replicates.

30. The vector of claim 29, wherein the vector is a conditionally replicating human immunodeficiency virus (crHIV) vector.

31. The vector of claim 25, wherein the promoter and/or enhancer is macrophage-specific.

32. The vector of claim 25, wherein the promoter and/or enhancer is tumor-specific.

33. The method of claim 20, wherein the heterologous nucleic acid sequence is a sequence transcribed to an RNA selected from the group consisting of an antisense molecule, an RNA decoy, and a ribozyme, or the heterologous nucleic acid sequence encodes a protein selected from the group consisting of a transdominant mutant, a toxin, and a single-chain antibody (scAb) directed to a viral structural protein.

34. A method of secreting an antibody in a cell comprising: (a) contacting a cell with a helper vector comprising a promoter and/or enhancer that can be activated by a cellular factor present in the cell; and (b) contacting the cell with a **retroviral** vector comprising 5' and 3' long terminal repeats (LTRs) derived from a **lentivirus**, and a heterologous nucleic acid sequence operably linked 3' of the 5' LTR to permit its expression by the 5' LTR, wherein said heterologous sequence encodes an antibody that is secreted and that acts upon the cellular factor, and wherein said 3' LTR is downstream of the heterologous

35. The method of claim 34, wherein the **lentivirus** is human immunodeficiency virus-1 (HIV-1) or human immunodeficiency virus-2 (HIV-2).

36. The method of claim 34, wherein the vector conditionally replicates.

37. The method of claim 36, wherein the vector is a conditionally replicating human immunodeficiency virus (crHIV) vector.

38. The method of claim 34, wherein the cellular factor is selected from the group consisting of a bacteria, a cytokine, an antibiotic, and a toxin.

39. The method of claim 34, wherein the heterologous sequence encodes an antibody selected from the group consisting of an anti-bacterial, anti-toxin, and anti-pathogenic antibody.

40. A **retroviral** vector comprising: (a) 5' and 3' long terminal repeats (LTRs) derived from a **lentivirus**, wherein the 5' LTR comprises a promoter and/or enhancer that is responsive to a cellular factor; and (b) a heterologous nucleic acid sequence operably linked 3' of the 5' LTR to permit its expression by the 5' LTR, wherein said heterologous sequence encodes an antibody that acts upon the cellular factor and wherein the 3' LTR is downstream of the heterologous sequence.

41. The **retroviral** vector of claim 40, wherein the **lentivirus** is human immunodeficiency virus-1 (HIV-1) or human immunodeficiency virus-2 (HIV-2).

42. The **retroviral** vector of claim 40, wherein the vector conditionally replicates.

43. The **retroviral** vector of claim 42, wherein the vector is a conditionally replicating human immunodeficiency virus (crHIV) vector.

44. The vector of claim 40, wherein the cellular factor is selected from the group consisting of a bacteria, a cytokine, an antibiotic, and a toxin.

45. The vector of claim 40, wherein the heterologous sequence encodes an antibody selected from the group consisting of an anti-bacterial, anti-toxin, and anti-pathogenic antibody.

46. The vector of claim 40, wherein the promoter is a tet promoter, a toxin responsive sequence, or a cytokine responsive promoter.

47. A **retroviral** vector comprising (a) 5' and 3' long terminal repeats (LTRs) derived from a **retrovirus**, wherein the 5' LTR comprises sequences that are not derived from said **retrovirus**; and (c) a heterologous nucleic acid sequence operably linked 3' of the 5' LTR to permit its expression by the 5' LTR, wherein said heterologous nucleic acid sequence is a genetic antiviral agent that is a siRNA and wherein the 3' LTR is downstream of the heterologous sequence.

48. A method of expressing a heterologous nucleic acid sequence in a specific cell comprising (a) contacting said cell with a **retroviral** vector comprising 5' and 3' long terminal repeats (LTRs) derived from a **retrovirus**, a heterologous nucleic acid sequence operably linked 3' of the 5' LTR to permit its expression by the 5' LTR, wherein said heterologous nucleic acid sequence is a genetic antiviral agent that is a siRNA, wherein the 3' LTR is downstream of the heterologous sequence, and lacking a nucleotide sequence encoding Tat, and (b) contacting said cell with a helper vector comprising a nucleotide sequence encoding Tat, and a cell specific promoter and/or enhancer that is capable of driving the expression of the Tat gene.

L15 ANSWER 4 OF 12 USPATFULL on STN
2003:127206 Regulated nucleic acid expression system.
Chang, Yung-Nien, Cockeysville, MD, UNITED STATES
Ni, Yajin, Germantown, MD, UNITED STATES
Dropulic, Boro, Ellicott City, MD, UNITED STATES
US 2003087419 A1 20030508
APPLICATION: US 2001-20472 A1 20011030 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides nucleic acid constructs, expression systems, and methods relating to the regulation of gene expression. The invention may be applied to regulate the expression of any coding sequence of interest, including those coding for viral components necessary for the packaging of viral particles.

1. A packaging cell comprising a first, second, and third nucleic acid constructs which regulate expression of one or more than one viral gene product necessary for packaging a viral vector wherein said first nucleic construct is capable of expressing an encoded first product; said first product is capable of regulating expression of a second product encoded on said second nucleic acid construct; and said second product is capable of regulating expression of said viral gene product, which is encoded by a sequence present on said third nucleic acid construct.

2. The cell of claim 1 wherein said first nucleic acid construct comprises a tetracycline regulated promoter/operator.

3. The cell of claim 1 wherein said first product is a transactivator of a tetracycline regulated promoter/operator or a fusion protein comprising said transactivator.

4. The cell of claim 1 wherein said second nucleic acid construct comprises a tetracycline regulated promoter/operator.

5. The cell of claim 1 wherein said second product is a rev protein.

6. The cell of claim 1 wherein said third construct comprises a promoter derived from a **retroviral** 5' LTR.

7. The cell of claim 1 wherein said viral gene product is a viral envelope or G protein.

8. The cell of claim 7 further comprising an additional nucleic acid construct that encodes **retroviral** gag and pol proteins.

9. The cell of claim 1 wherein said first product is tat protein or a chimeric protein comprising a tat protein.

10. The cell of claim 7 wherein said viral gene product is a G protein.

11. The cell of claim 1 which is stably transfected with said nucleic acid constructs.

12. The cell of claim 1 further comprising a conditionally replicating viral vector and wherein said cell packages said vector.

13. The cell of claim 12 wherein said vector is derived from HIV-1.

14. The cell of claim 13 wherein said G protein is a VSV or Mokola virus G protein.

15. A method of packaging a viral vector comprising culturing the cell of claim 13 under conditions wherein said first nucleic acid construct expresses said first product.

L15 ANSWER 5 OF 12 USPATFULL on STN
2002:340252 **Lentiviral** vectors.
Dropulic, Boro, Ellicott City, MD, United States
Pitha-Rowe, Paula, Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 6498033 B1 20021224
APPLICATION: US 2000-524004 20000313 (9)
PRIORITY: US 1993-32800P 19931128 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.
CLM What is claimed is:
1. A **retroviral** vector comprising 5' and 3' long terminal repeats (LTRs) derived from a **Lentivirus,** a truncated gag gene sequence containing the **Lentiviral** packaging signal, and a heterologous nucleic acid sequence operably linked 3' of the truncated gag gene sequence to permit its expression by the 5' LTR.

2. The vector of claim 1 wherein said truncated gag gene lacks sequences

pNL4-3.

3. The vector of claim 1 wherein said vector lacks **Lentiviral** sequences from the truncated gag gene to within the nef gene.

4. The vector of claim 1 wherein said **Lentivirus** is HIV-1 or HIV-2.

5. The vector of claim 4 wherein said **Lentivirus** is HIV-1.

6. The vector of claim 1 wherein said heterologous nucleic acid sequence is or encodes a genetic antiviral agent.

7. The vector of claim 1 wherein said genetic antiviral agent is selected from the group consisting of antisense molecules, RNA decoys, transdominant mutants, toxins, single-chain antibodies (scAbs) directed to a viral structural protein, and ribozymes.

8. The vector of claim 7 wherein said genetic antiviral agent is a ribozymne.

9. The vector of claim 1 further comprising a promoter 3' of said heterologous nucleic acid sequence.

10. The vector of claim 1 further comprising an inserted rev responsive element (RRE).

L15 ANSWER 6 OF 12 USPATFULL on STN
2002:152407 Methods to assay gene function with viral vectors.
Dropulic, Boro, Ellicott City, MD, United States
Pitha-Rowe, Paula, Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 6410257 B1 20020625
APPLICATION: US 2000-562894 20000501 (9)
PRIORITY: US 1995-32800P 19951128 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to vital vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.

CLM What is claimed is:
1. A method of determining the functional properties of a cellular gene product, which method comprises: a) contacting a host cell with a vector comprising at least a first and second nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, a ribozyme capable of cleaving native RNA encoding said cellular gene product; (ii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iii) the vector is selectively replicated over said helper virus or helper vector; and (iv) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence; b) expressing said first nucleotide sequence in the host cell; and c) determining the functional properties of the cellular gene product.

2. The method of claim 1 wherein said vector is a **retroviral** vector.

3. The method of claim 1 wherein said helper virus or helper vector comprises a nucleotide sequence that further reduces genetic recombination between said vector and said helper virus or helper vector.

4. The method of claim 3 wherein said nucleotide sequence present in said helper virus or helper vector is different from said second nucleotide sequence.

5. The method of claim 1, wherein said second nucleotide sequence comprises a genetic antiviral agent.

6. The method of claim 5, wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, a cellular antigen or receptor.

7. The method of claim 6, wherein said genetic antiviral agent is a hammerhead or external guide sequence (EGS) type ribozyme.

8. The method of claim 1, wherein said vector further comprises a selectable marker.

9. The method of claim 1 wherein said determining includes screening for factors that interact with said gene product or for activities associated with said gene product.

10. A vector for determining the functional properties of a gene product, which vector comprises at least a first and second nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, a ribozyme capable of cleaving native RNA encoding said cellular gene product; (ii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iii) the vector is selectively replicated over said helper virus or helper vector; and (iv) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence.

11. A method of assaying for a functional property of a gene product, which method comprises: a) contacting a host cell with a conditionally replicating vector comprising at least a first nucleotide sequence capable of expressing said gene product and a second nucleotide that comprises, or encodes, in which case it also expresses, a genetic antiviral agent selected from an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant protein, a single chain antibody, a cytokine, a cellular antigen or receptor; b) expressing said gene product in the host cell; and c) assaying for the functional properties of the expressed gene product.

12. The method of claim 11 wherein said vector is a **retroviral** vector.

13. The method of claim 11 wherein said genetic antiviral agent is a hammerhead or external guide sequence (EGS) type ribozyme.

14. The method of claim 11, wherein said vector further comprises a selectable marker.

15. The method of claim 11 wherein said assaying includes screening for factors that interact with said gene product or for activities associated with said gene product.

16. A method of determining the functional properties of a cellular gene product, which method comprises: a) contacting a host cell with a vector comprising at least a first, second and third nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, a ribozyme capable of cleaving native RNA encoding said cellular gene product; (ii) said third nucleotide sequence is capable of expressing a mutagenized form of said cellular gene product and is resistant to said first nucleotide sequence; (iii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iv) the vector is selectively replicated over said helper virus or helper vector; and (v) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence; b) expressing said first and third nucleotide sequences in the host cell; and c) determining the functional properties of the cellular gene product.

17. The method of claim 16 wherein said vector is a **retroviral** vector.

18. The method of claim 16 wherein said helper virus or helper vector comprises a nucleotide sequence that further reduces genetic recombination between said vector and said helper virus or helper vector.

19. The method of claim 18 wherein said nucleotide sequence present in said helper virus or helper vector is different from said second nucleotide sequence.

20. The method of claim 16, wherein said second nucleotide sequence comprises a genetic antiviral agent.

21. The method of claim 20, wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, a cellular antigen or receptor.

22. The method of claim 21, wherein said genetic antiviral agent is a hammerhead or external guide sequence (EGS) type ribozyme.

23. The method of claim 16, wherein said vector further comprises a selectable marker.

24. The method of claim 16 wherein said third nucleotide sequence contains silent point mutations that impart resistance to said first nucleotide sequence.

25. The method of claim 16 wherein said determining includes screening for factors that interact with said gene product or for activities associated with said gene product.

26. A vector for determining the functional properties of a gene product, which vector comprises at least a first, second and third nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, a ribozyme capable of cleaving native RNA encoding said cellular gene product; (ii) said third nucleotide sequence is capable of expressing a mutagenized form of said cellular gene product and is resistant to said first nucleotide sequence; (iii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iv) the vector is selectively replicated over said helper virus or helper vector; and (v) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence.

27. A method of assaying for a functional property of a gene product, which method comprises: a) contacting a host cell with a vector comprising at least a first nucleotide sequence capable of expressing said gene product and a second nucleotide sequence wherein: (i) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (ii) the vector is selectively replicated over said helper virus or helper vector; and (iii) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence; b) expressing said gene product in the host cell; and c) assaying for the functional properties of the expressed gene product.

28. The method of claim 27 wherein said vector is a **retroviral** vector.

29. The method of claim 27 wherein said helper virus or helper vector comprises a nucleotide sequence that further reduces genetic recombination between said vector and said helper virus or helper vector.

30. The method of claim 29 wherein said nucleotide sequence present in said helper virus or helper vector is different from said first nucleotide sequence.

31. The method of claim 27, wherein said second nucleotide sequence comprises or encodes, in which case it also expresses, a genetic antiviral agent.

32. The method of claim 31, wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, a cellular antigen or receptor.

33. The method of claim 32, wherein said genetic antiviral agent is a hammerhead or external guide sequence (EGS) type ribozyme.

34. The method of claim 27, wherein said vector further comprises a selectable marker.

35. The method of claim 27 wherein said assaying includes screening for factors that interact with said gene product or for activities associated with said gene product.

36. A vector for assaying for the functional properties of a gene product, which vector comprises: at least a first nucleotide sequence capable of expressing said gene product and a second nucleotide sequence wherein: (i) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (ii) the vector is selectively replicated over said helper virus or helper vector; and (iii) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence.

37. A method of determining the functional properties of a cellular gene product, which method comprises: a) contacting a host cell with a vector comprising at least a first and second nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, an agent selected from an antisense molecule, a

single chain antibody capable of inhibiting expression of native RNA encoding said cellular gene product; (ii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iii) the vector is selectively replicated over said helper virus or helper vector; and (iv) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence; b) expressing said first nucleotide sequence in the host cell; and c) determining the functional properties of the cellular gene product.

38. The method of claim 37 wherein said vector is a **retroviral** vector.

39. The method of claim 37 wherein said helper virus or helper vector comprises a nucleotide sequence that further reduces genetic recombination between said vector and said helper virus or helper vector.

40. The method of claim 39 wherein said nucleotide sequence present in said helper virus or helper vector is different from said second nucleotide sequence.

41. The method of claim 37, wherein said vector further comprises a selectable marker.

42. The method of claim 37 wherein said determining includes screening for factors that interact with said gene product or for activities associated with said gene product.

43. A vector for determining the functional properties of a gene product, which vector comprises at least a first and second nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, an agent selected from an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein or a single chain antibody capable of inhibiting expression of native RNA encoding said cellular gene product; (ii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iii) the vector is selectively replicated over said helper virus or helper vector; and (iv) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence.

44. A method of determining the functional properties of a cellular gene product, which method comprises: a) contacting a host cell with a vector comprising at least a first, second and third nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, an agent selected from an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein or a single chain antibody capable of inhibiting expression of native RNA encoding said cellular gene product; (ii) said third nucleotide sequence is capable of expressing a mutagenized form of said cellular gene product and is resistant to said first nucleotide sequence; (iii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iv) the vector is selectively replicated over said helper virus or helper vector; and (v) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence; b) expressing said first and third nucleotide sequences in the host cell; and c) determining the functional properties of the cellular gene product.

45. The method of claim 44 wherein said vector is a **retroviral** vector.

46. The method of claim 44 wherein said helper virus or helper vector comprises a nucleotide sequence that further reduces genetic recombination between said vector and said helper virus or helper vector.

47. The method of claim 46 wherein said nucleotide sequence present in said helper virus or helper vector is different from said second nucleotide sequence.

48. The method of claim 44, wherein said vector further comprises a selectable marker.

49. The method of claim 44 wherein said third nucleotide sequence contains silent point mutations that impart resistance to said first nucleotide sequence.

50. The method of claim 44 wherein said determining includes screening for factors that interact with said gene product or for activities associated with said gene product.

product, which vector comprises at least a first, second and third nucleotide sequences wherein: (i) said first nucleotide sequence comprises or encodes, in which case it also expresses, an agent selected from an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant protein or a single chain antibody capable of inhibiting expression of native RNA encoding said cellular gene product; (ii) said third nucleotide sequence is capable of expressing a mutagenized form of said cellular gene product and is resistant to said first nucleotide sequence; (iii) the vector replicates in said host cell only upon complementation with a helper virus or a helper vector; (iv) the vector is selectively replicated over said helper virus or helper vector; and (v) genetic recombination between said vector and said helper virus or helper vector is reduced by the presence of said second nucleotide sequence.

L15 ANSWER 7 OF 12 USPATFULL on STN
2001:71355 Methods to inhibit replication of infective virus.
Dropulic, Boro, Ellicott City, MD, United States
Pitha, Paula M., Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 6232120 B1 20010515
APPLICATION: US 1999-251283 19990216 (9)
PRIORITY: US 1995-32800P 19951128 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.

CLM What is claimed is:

1. A method to inhibit the replication of an infective replicable human immunodeficiency virus (HIV) in a cell, which method comprises contacting the cell, which is infected or at risk for being infected with said HIV, with a conditionally replicating human immunodeficiency viral vector which comprises at least a first nucleotide sequence, wherein said contacting occurs ex vivo or in vitro, and wherein said first nucleotide sequence adversely affects said HIV; and wherein the conditionally replicating human immunodeficiency viral vector replicates in a host cell only upon complementation with a wild-type virus or a helper virus, or a helper vector, and wherein said complementation renders the host cell permissive for replication of said conditionally replicating vector; and wherein said vector is selectively replicated over said wild-type virus, helper virus, or helper vector.

2. The method of claim 1 wherein the presence of said first nucleotide sequence inhibits replication of said infective replicable HIV in said cell.

3. The method of claim 2 wherein said first nucleotide sequence comprises or encodes, in which case it also expresses, a genetic antiviral agent.

4. The method of claim 3 wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, a cellular antigen or receptor.

5. The method of claim 4 wherein said genetic antiviral agent is a transdominant mutant protein.

6. The method of claim 4 wherein said genetic antiviral agent is a ribozyme.

7. The method of claim 6, wherein said ribozyme is in a ribozyme cassette comprising one, two or multiple ribozymes.

8. The method of claim 7, wherein each ribozyme of said cassette cleaves a different site or the same site.

9. The method of claim 7, wherein said ribozyme cassette comprises two or three ribozymes.

10. The method of claim 4 wherein said genetic antiviral agent encodes a

11. The method of claim 4 wherein said genetic antiviral agent is an antisense molecule.

12. The method of claim 1 wherein said conditionally replicating human immunodeficiency viral vector comprises at least one second nucleotide sequence, which confers to said host cell a selective advantage over a second cell infected with a wild-type strain of virus or helper virus or helper vector, but wherein said second cell lacks said conditionally replicating human immunodeficiency viral vector, or confers a selective advantage to said conditionally replicating human immunodeficiency viral vector over said wild-type strain, helper virus or helper vector.

13. The method of claim 12 wherein said second nucleotide sequence confers multidrug resistance, encodes a mutant protease, encodes a mutant reverse transcriptase or comprises a promoter, optionally including an enhancer, that is activated in said host cell in preference to promoters present in said wild-type virus strain, helper virus or helper vector.

14. The method of claim 13, wherein said second nucleotide sequence confers multidrug resistance and said conditionally replicating human immunodeficiency viral vector is used with a drug.

15. The method of claim 13, wherein said second nucleotide sequence comprises a promoter, optionally including an enhancer, that is preferentially activated in said host cell and said conditionally replicating human immunodeficiency viral vector is used with a cytokine.

16. The method of claim 1 wherein said infective replicable HIV virus causes AIDS.

17. The method of claim 1 wherein said vector is packaged in an infectious viral particle or formulated in a liposome.

18. The method of claim 1 wherein said conditionally replicating human immunodeficiency viral vector is a chimeric vector comprising sequences derived from different viruses.

19. The method of claim 18 wherein said chimeric vector comprises sequences derived from HIV, adenovirus, adeno-associated virus, Sindbis virus, or combinations thereof.

20. The method of claim 18 wherein said cell is of hematopoietic origin.

21. The method of claim 18 wherein said first nucleotide sequence is derived from a wild-type HIV.

22. The method of claim 18 wherein said vector is packaged in an infectious viral particle or formulated in a liposome or with an adjuvant.

23. The method of claim 18, wherein said contacting occurs ex vivo.

24. The method of claim 23, further comprising return of said cell to an in vivo location after said ex vivo contacting.

25. The method of claim 1 wherein said conditionally replicating human immunodeficiency viral vector comprises sequences derived from HIV-1 and HIV-2.

26. The method of claim 25 wherein said infective replicable human immunodeficiency virus is HIV-1 or HIV-2.

27. The method of claim 1 wherein said infective replicable human immunodeficiency virus is HIV-1.

28. The method of claim 1 wherein said cell is of hematopoietic origin.

29. The method of claim 1 wherein said first nucleotide sequence is derived from a wild-type HIV.

30. The method of claim 29, further comprising return of said cell to an in vivo location after said ex vivo contacting.

31. The method of claim 1 wherein said vector is packaged in an infectious viral particle or formulated in a liposome or with an adjuvant.

32. The method of claim 1, wherein said contacting occurs ex vivo.

2001:43984 Conditionally replicating viral vectors and their use.
Dropulic, Boro, Ellicott City, MD, United States
Pitha, Paula M., Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 6207426 B1 20010327
APPLICATION: US 1999-251084 19990216 (9)
PRIORITY: US 1995-32800P 19951128 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.
CLM What is claimed is:
1. A method to prepare a conditionally replicating virus product vector, which method comprises: (a) obtaining a starting vector, which replicates only in a host cell that is permissive for replication of said starting vector; and (b) incorporating into the starting vector at least a first nucleotide sequence which adversely affects a wild-type virus, a helper virus, a helper vector, or wild-type mobile genetic element to obtain said product vector wherein said product vector is selectively replicated over a wild-type virus, a helper virus, a helper vector or mobile genetic element wherein said host cell is permissive by virtue of the presence of a wild-type virus, or helper virus, helper vector or wild-type mobile genetic element.

2. The method of claim 1 wherein said first nucleotide sequence comprises or encodes, in which case it also expresses, a genetic antiviral agent.

3. The method of claim 1 wherein said first nucleotide sequence is a genetic antiviral agent.

4. The method of claim 2 wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, a cellular antigen or receptor.

5. The method of claim 4 wherein said ribozyme is in a ribozyme cassette comprising one, two or multiple ribozymes.

6. The method of claim 5 wherein each ribozyme of said cassette cleaves a different site.

7. The method of claim 5 wherein said ribozyme cassette comprises at least 3 ribozymes.

8. The method of claim 3 wherein said genetic antiviral agent encodes a single-chain antibody to a protein of a wild-type virus, helper virus or helper vector.

9. The method of claim 3 wherein said genetic antiviral agent is an antisense molecule.

10. The method of claim 1 wherein said conditionally replicating vector comprises at least one second nucleotide sequence, which confers to said host cell a selective advantage over a second cell infected with a wild-type strain of virus or helper virus or helper vector, but wherein said second cell lacks said conditionally replicating vector, or confers a selective advantage to said conditionally replicating vector over said wild-type strain, helper virus or helper vector.

11. The method of claim 10 wherein said second nucleotide sequence confers multidrug resistance, encodes a mutant protease, encodes a mutant reverse transcriptase, or comprises a promoter, optionally including an enhancer, that is activated in said host cell in preference to promoters present in said wild-type virus strain, helper virus or helper vector.

12. The method of claim 11, wherein said second nucleotide sequence confers multidrug resistance and said conditionally replicating vector is used with a drug.

13. The method of claim 11, wherein said second nucleotide sequence

preferentially activated in said host cell and said conditionally replicating vector is used with a cytokine.

14. The method of claim 1 wherein the virus product vector, wild-type virus, or helper virus comprises an RNA genome.

15. The method of claim 1 wherein the virus product vector, wild-type virus, or helper virus is a member of the family Coronaviridae, Flaviviridae, Picornaviridae, Togaviridae, Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Filoviridae, Bunyaviridae, **Retroviridae**, Hependaviridae or Reoviridae.

16. The method of claim 15 wherein the virus product vector, wild-type virus, or helper virus is a member of the family **Retroviridae**.

17. The method of claim 16 wherein said virus product vector is selectively replicated over a wild-type virus, a helper virus, a helper vector or mobile genetic element in a host cell selected from the group consisting of a hematopoietic stem cell, a fibroblast cell, an epithelial cell, a blood or blood vessel cell, a cell from the respiratory system, a cell from the gastrointestinal system, a cell from the urinary system, a cell from the nervous system, a cell from the integumentary system, and an antigen presenting cell.

18. The method of claim 17 wherein said host cell is selected from the group consisting of a lymphocyte, a macrophage, and an astrocyte.

19. The method of claim 1 wherein said first nucleotide sequence is derived from a wild-type virus.

L15 ANSWER 9 OF 12 USPATFULL on STN
2001:1641 Genetic antiviral agents and methods for their use.
Dropulic, **Boro**, Ellicott City, MD, United States
Pitha, Paula M., Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 6168953 B1 20010102
APPLICATION: US 1999-312322 19990514 (9)
PRIORITY: US 1995-32800P 19951128 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.
CLM What is claimed is:
1. A method of increasing the efficiency of a genetic antiviral agent in a host cell comprising contacting, in a host cell, a target with a genetic antiviral agent which has been incorporated into a vector, wherein said vector contains at least one cis-acting genetic element colocalizing the genetic antiviral agent with said target, said vector is selectively replicated over said target, and said vector is derived from a virus of a genus or subfamily selected from the group consisting of Spumavirinae, Spumavirus, **Lentivirinae**, and **Lentivirus**.

2. The method of claim 1 wherein said target is RNA.

3. The method of claim 1 wherein said target is a wild-type virus, a helper virus, a helper vector or a mobile genetic element.

4. The method of claim 3 wherein said vector is derived from a wild-type human immunodeficiency virus.

5. The method of claim 1 wherein said vector is derived from a **Lentivirus**.

6. The method of claim 5 wherein said vector is derived from a wild-type human immunodeficiency virus.

7. The method of claim 1 wherein said vector is a conditionally replicating vector.

8. The method of claim 2 wherein said genetic antiviral agent comprises a nucleic acid sequence, which comprises or encodes, in which case it

9. The method of claim 8 wherein said ribozyme is in a ribozyme cassette comprising one, two or multiple ribozymes.

10. The method of claim 9 wherein each ribozyme of said cassette cleaves a different site.

11. The method of claim 9 wherein said ribozyme cassette comprises at least 3 ribozymes.

12. The method of claim 1 wherein said genetic element is a packaging, export, or dimerization signal.

13. The method of claim 12 wherein said genetic element is a packaging or dimerization signal.

14. The method of claim 1 wherein said vector is resistant to the genetic antiviral agent.

15. A genetic antiviral agent incorporated into a vector which contains at least one cis-acting genetic element colocalizing the genetic antiviral agent with the target of said agent, and said vector is derived from a virus of a genus or subfamily selected from the group consisting of Spumavirinae, Spumavirus, **Lentivirinae**, and **Lentivirus**, wherein said vector is selectively replicated over said target in a host cell.

16. The method of claim 15 wherein said target is a wild-type virus, a helper virus, a helper vector or a mobile genetic element.

17. The agent of claim 16 wherein said target is a wild-type human immunodeficiency virus.

18. The agent of claim 15 wherein said target is RNA.

19. The agent of claim 18 wherein said agent comprises a nucleic acid sequence, which comprises or encodes, in which case it also expresses, a ribozyme which cleaves said target RNA.

20. The agent of claim 19 wherein said ribozyme is in a ribozyme cassette comprising one, two or multiple ribozymes.

21. The agent of claim 20 wherein each ribozyme of said cassette cleaves a different site.

22. The agent of claim 20 wherein said ribozyme cassette comprises at least 3 ribozymes.

23. The agent of claim 15 wherein said vector is derived from a **Lentivirus**.

24. The agent of claim 23 wherein said vector is derived from a wild-type human immunodeficiency virus.

25. The agent of claim 15 wherein said vector is a conditionally replicating vector.

26. The agent of claim 15 wherein said genetic element is a packaging, export, stabilizing, or dimerization signal.

27. The agent of claim 26 wherein said genetic element is a packaging or dimerization signal.

28. The agent of claim 15 wherein said vector is resistant to the genetic antiviral agent.

L15 ANSWER 10 OF 12 USPATFULL on STN
2000:117522 Methods to express genes from viral vectors.
Dropulic, Boro, Ellicott City, MD, United States
Pitha, Paula M., Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 6114141 20000905
APPLICATION: US 1999-251085 19990216 (9)
PRIORITY: US 1995-32800P 19951128 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical

host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.

CLM What is claimed is:

1. A method of expressing a gene in a host cell, which method comprises: a) contacting the host cell with: (i) a conditionally replicating virus vector which comprises at least one gene to be expressed and further comprises at least a first nucleotide sequence wherein: (a) the conditionally replicating vector replicates in said host cell only upon complementation with a wild-type virus, a helper virus, or a helper vector; and (b) the conditionally replicating vector is selectively replicated over said wild-type virus, helper virus, or helper vector; and (ii) a wild-type virus, a helper virus, or a helper vector, wherein said wild-type virus, helper virus or helper vector is adversely affected by the presence of said first nucleotide sequence; or with: (i) a helper virus or a helper vector, which comprises at least a first nucleotide sequence, wherein said helper virus or helper vector is not adversely affected by the presence of said first nucleotide sequence; and (ii) a conditionally replicating virus vector which comprises at least one gene to be expressed wherein: (a) the conditionally replicating vector replicates in a host cell only upon complementation with a wild-type virus, a helper virus, or a helper vector; (b) genetic recombination between the conditionally replicating vector and the helper virus or helper vector is reduced by the presence of said first nucleotide sequence; and (c) the conditionally replicated vector is selectively replicated over said helper virus or helper vector; and b) expressing said gene in the host cell or in a cell infected with a vector replicated from said conditionally replicating vector in said host cell.

2. The method of claim 1 wherein expressing said gene in said host cell inhibits replication of a wild-type strain of said virus in said cell.

3. The method of claim 1, wherein said first nucleotide sequence comprises or encodes, in which case it also expresses, a genetic antiviral agent.

4. The method of claim 3, wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, a cellular antigen or receptor.

5. The method of claim 4, wherein said genetic antiviral agent comprises a ribozyme.

6. The method of claim 5 wherein said ribozyme is in a ribozyme cassette comprising one, two or multiple ribozymes.

7. The method of claim 6, wherein each ribozyme of said cassette cleaves a different site.

8. The method of claim 6 wherein said ribozyme cassette comprises at least two ribozymes.

9. The method of claim 4, wherein said genetic antiviral agent encodes a single-chain antibody to a protein of a wild-type virus, helper virus or helper vector.

10. The method of claim 4, wherein said genetic antiviral agent is an antisense molecule.

11. The method of claim 1, wherein said conditionally replicating vector comprises at least one second nucleotide sequence, which confers to said host cell a selective advantage over a second cell infected with a wild-type strain of virus or helper virus or helper vector, but wherein said second cell lacks said conditionally replicating vector, or confers a selective advantage to said conditionally replicating vector over said wild-type strain, helper virus or helper vector.

12. The method of claim 11, wherein said second nucleotide sequence confers multidrug resistance, encodes a mutant protease, encodes a mutant reverse transcriptase, or comprises a promoter, optionally including an enhancer, that is activated in said host cell in preference to promoters present in said wild-type virus strain, helper virus or helper vector.

13. The method of claim 11, wherein said second nucleotide sequence confers multidrug resistance and said conditionally replicating vector is used with a drug.

14. The method of claim 11, wherein said second nucleotide sequence comprises a promoter optionally, including an enhancer, that is preferentially activated in said host cell and said conditionally replicating vector is used with a cytokine.

15. The method of claim 1 wherein said conditionally replicating vector is selectively replicated over a wild-type virus, a helper virus, or a helper vector in a host cell selected from the group consisting of a hematopoietic stem cell, a fibroblast cell, an epithelial cell, a blood or blood vessel cell, a cell from the respiratory system, a cell from the gastrointestinal system, a cell from the urinary system, a cell from the nervous system, a cell from the integumentary system, and an antigen presenting cell.

16. The method of claim 15 wherein said host cell is selected from the group consisting of a lymphocyte, a macrophage, and an astrocyte.

17. The method of claim 1 wherein said first nucleotide sequence is derived from a wild-type virus.

18. A method of expressing a gene in a target cell, which method comprises: a) contacting the target cell with a replicated vector that has been produced in a host cell provided with: (i) a conditionally replicating virus vector which comprises at least one gene to be expressed and further comprises at least a first nucleotide sequence wherein: (a) the conditionally replicating vector is replicated in said host cell only upon complementation with a wild-type virus, a helper virus, or a helper vector; and (b) the conditionally replicating vector is selectively replicated over said wild-type virus, helper virus, or helper vector; and (ii) a wild-type virus, a helper virus, or a helper vector, wherein said wild-type virus, helper virus or helper vector is adversely affected by the presence of said first nucleotide sequence; or with: (i) a helper virus or a helper vector, which comprises at least a first nucleotide sequence, wherein said helper virus or helper vector is not adversely affected by the presence of said first nucleotide sequence; and (ii) a conditionally replicating virus vector which comprises at least one gene to be expressed wherein: (a) the conditionally replicating vector replicates in a host cell only upon complementation with a wild-type virus, a helper virus, or a helper vector; and (b) genetic recombination between the conditionally replicating vector and the helper virus or helper vector is reduced by the presence of said first nucleotide sequence; and (c) the conditionally replicated vector is selectively replicated over said helper virus or helper vector; and b) expressing said gene in the target cell.

19. The method of claim 18 wherein expressing said gene in said target cell inhibits replication of a wild-type strain of said virus in said cell.

20. The method of claim 18, wherein said first nucleotide sequence comprises or encodes, in which case it also expresses, a genetic antiviral agent.

21. The method of claim 20, wherein said genetic antiviral agent is an antisense molecule, a ribozyme, a nucleic acid decoy, a transdominant mutant protein, a single chain antibody, a cytokine, a cellular antigen or receptor.

22. The method of claim 21, wherein said genetic antiviral agent comprises a ribozyme.

23. The method of claim 22 wherein said ribozyme is in a ribozyme cassette comprising one, two or multiple ribozyymes.

24. The method of claim 23, wherein each ribozyme of said cassette cleaves a different site.

25. The method of claim 23 wherein said ribozyme cassette comprises at least two ribozymes.

26. The method of claim 21, wherein said genetic antiviral agent encodes a single-chain antibody to a protein of a wild-type virus, helper virus or helper vector.

27. The method of claim 21, wherein said genetic anti viral agent is an anti sense molecule.

28. The method of claim 18, wherein said conditionally replicating vector comprises at least one second nucleotide sequence, which confers to said target cell a selective advantage over a second cell infected with a wild-type strain of virus or helper virus or helper vector, but

confers a selective advantage to said conditionally replicating vector over said wild-type strain, helper virus or helper vector.

29. The method of claim 28, wherein said second nucleotide sequence confers multidrug resistance, encodes a mutant protease, encodes a mutant reverse transcriptase, or comprises a promoter optionally including an enhancer that is activated in said host cell in preference to promoters present in said wild-type virus strain, helper virus or helper vector.

30. The method of claim 28, wherein said second nucleotide sequence confers multidrug resistance and said conditionally replicating vector is used with a drug.

31. The method of claim 28, wherein said second nucleotide sequence comprises a promoter optionally including an enhancer that is preferentially activated in said host cell and said conditionally replicating vector is used with a cytokine.

32. The method of claim 18 wherein said conditionally replicating vector is selectively replicated over a wild-type virus, a helper virus, or a helper vector in a target cell selected from the group consisting of a hematopoictic stem cell, a fibroblast cell, an epithelial cell, a blood or blood vessel cell, a cell from the respiratory system, a cell from the gastrointestinal system, a cell from the urinary system, a cell from the nervous system, a cell from the integumentary system, and an antigen presenting cell.

33. The method of claim 32 wherein said target cell is selected from the group consisting of a lymphocyte, a macrophage, and an astrocyte.

34. The method of claim 18 wherein said first nucleotide sequence is derived from a wild-type virus.

L15 ANSWER 11 OF 12 USPATFULL on STN
1999:40189 Method of using a conditionally replicating viral vector to express a gene.
Dropulic , Boro, Ellicott City, MD, United States
Pitha, Paula M., Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 5888767 19990330
APPLICATION: US 1997-917625 19970822 (8)
PRIORITY: US 1995-32800P 19951125 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.
CLM What is claimed is:
1. A method of expressing a gene in a host cell, which method comprises: (A) contacting the host cell with: (i) a conditionally replicating human immunodeficiency viral vector which comprises the gene to be expressed and further comprises one or more first nucleotide sequences wherein: (a) the conditionally replicating vector replicates in a host cell only upon complementation with a wild-type strain of Human Immunodeficiency Virus (HIV), a helper virus, or a helper vector, each of which is sensitive to the presence of said one or more first nucleotide sequences; (b) the conditionally replicating vector is resistant to the presence of said one or more first nucleotide sequences; and (c) said one or more first nucleotide sequences causes selective packaging of the conditionally replicating vector into progeny virions over the wild-type strain of HIV, the helper virus, or the helper vector; and with (ii) the wild-type strain of HIV, the helper virus, or the helper vector; and (B) expressing said gene in the host cell.

2. The method of claim 1, wherein expressing said gene in said host cell inhibits replication of a wild-type strain of HIV in said cell.

3. The method of claim 1, wherein said one or more first nucleotide sequences comprises a genetic antiviral agent, which adversely affects the replication, expression, or replication and expression of a wild-type strain of HIV or of a helper virus or of a helper vector for

adversely affect said conditionally replicating vector, itself.

4. The method of claim 3, wherein said genetic antiviral agent is an antisense molecule.

5. The method of claim 3, wherein said genetic antiviral agent is a ribozyme.

6. The method of claim 5, wherein said ribozyme cleaves the nucleotide sequence of NUH.

7. The method of claim 5, wherein said ribozyme comprises the nucleotide sequence encoded by SEQ ID NO:3 or SEQ ID NO:4.

8. The method of claim 1, wherein said wild-type strain of HIV comprises the nucleotide sequence of SEQ ID NO:1 and said conditionally replicating viral vector is RNA and comprises a nucleotide sequence encoded by SEQ ID NO:2, 3, 4, 5, 6, 14, 15 or 16, wherein at least one N of SEQ ID NO: 14 is mutated.

9. The method of claim 1, wherein the conditionally replicating vector lacks the tat gene and its splice site from the genome of a wild-type strain of HIV.

10. The method of claim 9, wherein said conditionally replicating vector comprises a triple ribozyme cassette in place of said tat gene and its splice site, and wherein each ribozyme of the triple ribozyme cassette cleaves a different site on the genome of a wild-type strain of HIV.

11. The method of claim 10, wherein said wild-type strain of HIV comprises tat and each ribozyme of the triple ribozyme cassette cleaves a different site within tat.

12. The method of claim 1, wherein said wild-type strain of HIV comprises the nucleotide sequence of SEQ ID NO:1 and said conditionally replicating vector is DNA and comprises a nucleotide sequence of SEQ ID NO:2, 3, 4, 5, 6, 14, 15 or 16, wherein at least one N of SEQ ID NO:14 is mutated.

13. The method of claim 1, wherein said conditionally replicating vector comprises at least one second nucleotide sequence, which confers to said host cell a selective advantage over a second cell infected with a wild-type strain of HIV or with a helper virus or with a helper vector for replication of said conditionally replicating vector, but wherein said second cell lacks said conditionally replicating vector.

14. The method of claim 13, wherein said second one or more nucleotide sequences confers multidrug resistance or encodes a mutant protease or a mutant reverse transcriptase.

L15 ANSWER 12 OF 12 USPATFULL on STN
1999:36929 Methods to prepare conditionally replicating viral vectors.
Dropulic , Boro, Ellicott City, MD, United States
Pitha, Paula M., Baltimore, MD, United States
The Johns Hopkins University School of Medicine, Baltimore, MD, United States (U.S. corporation)
US 5885806 19990323
APPLICATION: US 1996-758598 19961127 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.
CLM What is claimed is:
1. A method of making a product human inmunodeficiency viral vector, which method comprises: (a) obtaining a starting vector, which is derived from a wild-type strain of human immunodeficiency virus and which replicates only in a host cell that is permissive for replication of said starting vector; and (b) incorporating into the starting vector of (a) a nucleic acid sequence, which comprises or encodes, in which case it also expresses, a genetic antiviral agent, and thereby, or in addition thereto, modifying the starting vector of (a) to obtain a product vector, which is resistant to said genetic antiviral agent,

over (i) a wild-type strain of human immunodeficiency virus, which is sensitive to said genetic antiviral agent, or (ii) a helper virus or a helper vector for replication of a human immunodeficiency viral vector, which is sensitive to said genetic antiviral agent, when (i) said wild-type strain of human immunodeficiency virus or (ii) said helper virus or said helper vector for replication of a human immunodeficiency viral vector is present in said host cell.

2. The method of claim 1, wherein said genetic antiviral agent is an antisense molecule.

3. The method of claim 1, wherein said genetic antiviral agent is a ribozyme.

4. The method of claim 3, wherein step (b) comprises: (i) incorporating into the starting vector of (a), wherein said vector is DNA, a nucleotide sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4; and (ii) modifying the starting vector of (a) to comprise a nucleotide sequence of SEQ ID NO:14, in which at least one N is mutated.

5. The method of claim 4, wherein step (b) comprises; (i) incorporating into the starting vector of (a) SEQ ID NO:3; and (ii) modifying the starting vector of (a) to comprise a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 5 and 15.

6. The method of claim 4, wherein step (b) comprises: (i) incorporating into the starting vector of (a) SEQ ID NO:4; and (ii) modifying the starting vector of (a) to comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 6 and 16.

7. The method of claim 3, wherein step (b) comprises: (i) incorporating into the starting vector of (a), wherein said vector is RNA, a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4; and (ii) modifying the starting vector of (a) to comprise a nucleotide sequence encoded by a nucleotide sequence of SEQ ID NO:14, in which at least one N is mutated.

8. The method of claim 7, wherein step (b) comprises: (i) incorporating into the starting vector of (a) the nucleotide sequence encoded by SEQ ID NO:3; and (ii) modifying the starting vector of (a) to comprise a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 5 and 15.

9. The method of claim 7, wherein step (b) comprises: (i) incorporating into the vector of (a) the nucleotide sequence encoded by SEQ ID NO:4;and (ii) modifying the starting vector of (a) to comprise a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 6 and 16.

10. The method of claim 3, wherein said ribozyme is a ribozyme cassette comprising one, two or multiple ribozymes.

11. The method of claim 10, wherein said ribozyme cassette comprises multiple ribozymes.

12. The method of claim 11, wherein said ribozyme cassette comprises three ribozymes.

13. A conditionally replicating human immunodeficiency viral vector, wherein said vector is selected from the group consisting of crHIV-1.1, crHIV-1.11, crHIV- 1.12, and crHIV-1.111.

14. A method of modifying a vector, which method comprises: (a) obtaining a human inmunodeficiency viral vector, wherein said vector is DNA; and (b) introducing into the vector of (a) a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 5, 6, 15 and 16.

15. An isolated and purified RNA molecule comprising a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 5, 6, 15 and 16.

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=> e asada kiyozo/in
E1          2     ASADA KIYOSHI/IN
E2          1     ASADA KIYOTOSHI/IN
E3         51 --> ASADA KIYOZO/IN
E4         14     ASADA KOHEI/IN
E5          1     ASADA KOHSEI/IN
E6          1     ASADA KOICHI/IN
E7          6     ASADA KOJI/IN
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E9            6     ASADA KOUJI/IN
E10           1     ASADA KOUJIROU/IN
E11           2     ASADA KUNIHIRO/IN
E12           1     ASADA KYOJI/IN

=> s e3
L16          51 "ASADA KIYOZO"/IN

=> s l16 and (retrovir? or lentivir?)
         41371 RETROVIR?
          6306 LENTIVIR?
L17         12 L16 AND (RETROVIR? OR LENTIVIR?)

=> d l17,ti,1-5

L17  ANSWER 1 OF 12  USPATFULL on STN
TI     Method for gene transfer into target cells with retrovirus

L17  ANSWER 2 OF 12  USPATFULL on STN
TI     Gene transfer method

L17  ANSWER 3 OF 12  USPATFULL on STN
TI     Method for amplifying nucleic acid sequence

L17  ANSWER 4 OF 12  USPATFULL on STN
TI     Method for amplifying nucleic acid sequence

L17  ANSWER 5 OF 12  USPATFULL on STN
TI     Gene transfer methods


=> d l17,cbib,ab,clm,1-12

L17  ANSWER 1 OF 12  USPATFULL on STN
2006:34275 Method for gene transfer into target cells with retrovirus.
    Asada, Kiyozo, Koka-gun, JAPAN
    Uemori, Takashi, Otsu-shi, JAPAN
    Ueno, Takashi, Kusatsu-shi, JAPAN
    Koyama, Nobuto, Uji-shi, JAPAN
    Hashino, Kimikazu, Takatsuki-shi, JAPAN
    Kato, Ikunoshin, Uji-shi, JAPAN
    Takara Bio Inc., a corporation of Japan, Otsu, JAPAN (non-U.S. corporation)
    US 2006030046 A1 20060209
    APPLICATION: US 2005-181091  A1  20050714 (11)
    PRIORITY: JP 1995-294382  19951113
    JP 1996-51847  19960308
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     A polypeptide represented by SEQ. ID No. 13, a polypeptide represented
       by SEQ. ID No. 30 or functional equivalents thereof and a polypeptide
       represented by SEQ. ID No. 17.
CLM    What is claimed is:
       1. In a method for increasing the efficiency of gene transfer into
       target cells with a retrovirus, wherein the improvement comprises
       carrying out the transduction by infecting the target cells with the
       retrovirus in the presence of a mixture of an effective amount of a
       functional material having retrovirus binding domain, and an effective
       amount of another functional material having target cell binding domain.

       2. A method according to claim 1, wherein the functional material having
       retrovirus binding domain is a functional material selected from
       Heparin-II binding region of fibronectin, fibroblast growth factors,
       collagens, polylysines and functional equivalents thereof.

       3. A method according to claim 1, wherein the functional material having
       target cell binding domain is a ligand which specifically binds to the
       target cells.

       4. A method according to claim 3, wherein the ligand is selected from
       cell adhesion proteins, hormones, cytokines, antibodies, sugar chains,
       carbohydrates and metabolites.

       5. A method according to claim 4, wherein the cell adhesion protein is a
       cell binding domain polypeptide of fibronectin.

       6. A method according to claim 5, wherein the cell binding domain
       polypeptide of fibronectin is a polypeptide of the binding domain to
       VLA-5 and/or VLA-4.

       7. A method according to claim 4, wherein the ligand is erythropoietin.

       8. A method according to any one of claims 1 to 7, wherein the
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9. A culture medium for target cells to be used for gene transfer into the target cells with a **retrovirus** which comprises a mixture of an effective amount of a functional material having **retrovirus** binding domain, and an effective amount of another functional material having target cell binding domain.

10. A culture medium according to claim 9, wherein the functional material having **retrovirus** binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

11. A culture medium according to claim 9, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

12. A culture medium according to claim 11, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

13. A culture medium according to claim 12, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

14. A culture medium according to claim 13, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

15. A culture medium according to claim 12, wherein the ligand is erythropoietin.

16. A culture medium according to any one of claims 9 to 15, wherein the functional materials are immobilized.

17. A method for localization of a **retrovirus** which comprises incubating a culture medium containing the **retrovirus** contacted with a mixture of an effective amount of a functional material having **retrovirus** binding domain, and an effective amount of another functional material having target cell binding domain.

18. A method for localization according to claim 17, wherein the functional material having **retrovirus** binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

19. A method for localization according to claim 17, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

20. A method for localization according to claim 19, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

21. A method for localization according to claim 20, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

22. A method for localization according to claim 21, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

23. A method for localization according to claim 20, wherein the ligand is erythropoietin.

24. A method for localization according to any one of claims 17 to 23, wherein the functional materials are immobilized.

25. A kit for carrying out **retrovirus**-mediated gene transfer into target cells, which comprises: (a) an effective amount of a functional material having **retrovirus** binding domain and/or an effective amount of another functional material having target cell binding domain; (b) an artificial substrate for incubating the **retrovirus** and the target cells; and (c) a target cell growth factor for pre-stimulating the target cells.

26. A kit according to claim 25, wherein the functional material having **retrovirus** binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

27. A kit according to claim 25, wherein the functional material having target cell binding domain is a ligand which specifically binds to the

28. A kit according to claim 27, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

29. A kit according to claim 27, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

30. A kit according to claim 29, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

31. A kit according to claim 28, wherein the ligand is erythropoietin.

32. A kit according to any one of claims 25 to 31, wherein the functional materials are immobilized.

33. A method for localization of a **retrovirus** comprising incubating a culture medium containing the **retrovirus** contacted with an effective amount of a functional material having a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine.

34. A method for localization according to claim 33, wherein the functional material is immobilized.

35. In a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**, wherein the improvement comprises carrying out the transduction by infecting the target cells with the **retrovirus** in the presence of an effective amount of a functional material having a target cell binding domain, and a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

36. A method according to claim 35, wherein the target cell binding domain is a ligand which specifically binds to the target cells.

37. A method according to claim 36, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

38. A method according to claim 37, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

39. A method according to claim 38, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

40. A method according to claim 37, wherein the ligand is erythropoietin.

41. A method according to claim 35, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

42. A method according to claim 35, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

43. A method according to claim 35, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalent of the fragment.

44. A method according to claim 43, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

45. A method according to claim 35, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

46. A method according to any one of claims 35 to 45, wherein the functional material is immobilized.

47. A method according to any one of claims 35 to 45, wherein the functional material is used without immobilization.

48. A culture medium for target cells to be used for gene transfer into the target cells with a **retrovirus** which comprises an effective amount

retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

49. A culture medium according to claim 48, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

50. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

51. A culture medium according to claim 48, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalents of the fragment.

52. A culture medium according to claim 48, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

53. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

54. A culture medium according to any one of claims 48 to 53, wherein the functional material is immobilized.

55. A method for localization of a **retrovirus** which comprises incubating a culture medium containing the **retrovirus** contacted with an effective amount of a functional material having a target cell binding domain, and a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

56. A method for localization according to claim 55, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalents of the factor.

57. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

58. A method for localization according to claim 55, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalent of the fragment.

59. A method for localization according to claim 58, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

60. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

61. A method for localization according to any one of claims 50 to 60, wherein the functional material is immobilized.

62. A kit for carrying out **retrovirus**-mediated gene transfer into target cells, which comprises: (a) an effective amount of a functional material having a target cell binding domain, and a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule; (b) an artificial substrate for incubating the **retrovirus** and the target cells; and (c) a target cell growth factor for pre-stimulating the target cells.

63. A kit according to claim 62, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

64. A kit according to claim 62, wherein the functional material is a

or 5 of the Sequence Listing.

65. A kit according to claim 62, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalents of the fragment.

66. A kit according to claim 65, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

67. A kit according to claim 62, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

68. A kit according to any one of claims 62 to 67, wherein the functional material is immobilized.

69. A method according to claim 8 or 46, wherein the functional materials are immobilized on beads.

70. A culture medium according to claim 16 or 54, the functional materials are immobilized on beads.

71. A method for immobilization according to claim 24, 34 or 61, wherein the functional materials are immobilized on beads.

72. A kit according to claim 32 or 68, wherein the functional materials are immobilized on beads.

73. In a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**, wherein the improvement comprises infecting the target cells with the **retrovirus** in the presence of an effective amount of a functional material selected from the group consisting of substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof which is immobilized on beads.

74. In a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**, wherein the improvement comprises infecting the target cells with the **retrovirus** in the presence of an effective amount of a functional material selected from the group consisting of substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof which is not immobilized.

75. A method according to any one of claims 1 to 8, 35 to 47, 69, 73 and 74, wherein the target cells are cells selected from stem cells, hematopoietic cells, non-adherent low density mononuclear cells, adherent cells, bone marrow cells, hematopoietic stem cells, peripheral blood stem cells, umbilical blood cells, fetal hematopoietic stem sells, embryoplastic stem cells, embryonic cells, primordial germ cell, oocyte, oogonia, ova, spermatocyte, sperm, CD 34+cells, C-kit+cells, multipotential hematopoietic progenitor cells, unipotential hematopoietic progenitor cells, erythrocyte precursor cells, lymphocytic precursor cells, mature blood cells, lymphocytes, B cells, T cells, fibroblasts, neuroblasts, nerve cells, endothelial cells, angio-endothelial cells, hepatic cells, myoblasts, skeletal muscle cells, smooth muscle cells, cancer cells, myeloma cells and leukemia cells.

76. A method according to any one of claims 1 to 8, 17 to 24, 33 to 47, 55 to 61, 69, 71 and 73 to 75, wherein the **retrovirus** includes an exogenous gene.

77. A method according to claim 76, wherein the **retrovirus** is a recombinant **retroviral** vector.

78. A method according to claim 76, wherein the **retrovirus** is a replication deficient recombinant **retroviral** vector.

79. Transformant cells obtained by a method according to any one of claims 1 to 8, 35 to 47, 69 and 73 to 78.

80. A method for cellular grafting comprising grafting the transformant cells obtained by a method according to claim 79 into a vertebrate animal.

81. A polypeptide represented by SEQ. ID 13 of the Sequence Listing.

82. A gene encoding the polypeptide according to claim 81.

83. A gene according to claim 82 which is represented by SEQ. ID No. 17

conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a **retrovirus**.

84. A polypeptide represented by SEQ. ID No. 30 of the Sequence Listing or functional equivalents thereof.

85. A gene encoding the polypeptide according to claim 84.

86. A gene according to claim 85 which is represented by SEQ. ID No. 33 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a **retrovirus**.

87. A polypeptide represented by SEQ. ID No. 5 of the Sequence Listing or functional equivalents thereof.

88. A gene encoding the polypeptide according to claim 87.

89. A gene according to claim 88 which is represented by SEQ. ID No. 26 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a **retrovirus**.

L17 ANSWER 2 OF 12 USPATFULL on STN
2006:30610 Gene transfer method.
Ueno, Takashi, Shiga, JAPAN
Matsumura, Hajime, Shiga, JAPAN
Tanaka, Keiji, Shiga, JAPAN
Iwasaki, Tomoko, Shiga, JAPAN
Ueno, Mitsuhiro, Shiga, JAPAN
Fujinaga, Kei, Hokkaido, JAPAN
Asada, Kiyozo, Shiga, JAPAN
Kato, Ikunoshin, Kyoto, JAPAN
Takara Bio Inc., Shiga, JAPAN (non-U.S. corporation)
US 6995010 B1 20060207
WO 2001032899 20010510
APPLICATION: US 2002-111708 20001023 (10)
WO 2000-JP7373 20001023 20020429 PCT 371 date
PRIORITY: JP 2002-308839 19991029
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method of transferring a foreign gene into cells, characterized by involving: the step of transferring into the cells with the use of an adenovirus vector, a first nucleic acid, which has a sequence provided with adeno-associated virus-origin ITRs in both sides of the target foreign gene to be transferred, and a second nucleic acid, which has an adeno-associated virus-origin rep gene and a promoter for expressing this gene and carries a stuffer sequence inserted thereinto sandwiched in two recombinase recognition sequences and located between the rep gene and the promoter; and the step of expressing the Rep protein under the action of recombinase in the cells obtained in the above step to thereby integrate the target foreign gene into the chromosomal DNA.
CLM What is claimed is:
1. A system for transferring a foreign gene into a cell, which contains: a. a combination of: 1. an adenoviral vector containing a first nucleic acid which has a sequence in which ITRs from adeno-associated virus are positioned on both sides of a gene of interest to be transferred; and 2. an adenoviral vector containing a second nucleic acid which has a rep gene from adeno-associated virus in which the expression of Rep52 protein and Rep40 protein are prevented by changing the 225th amino acid residue in Rep78 from methionine to glycine and a promoter for expressing the rep gene and in which a stuffer sequence put between two recombinase recognition sequences is inserted between the rep gene and the promoter; or b. an adenoviral vector containing: a first nucleic acid which has a sequence in which ITRs from adeno-associated virus are positioned on both sides of a gene of interest to be transferred; and a second nucleic acid which has a rep gene from adeno-associated virus and a promoter for expressing the rep gene and in which a stuffer sequence put between two recombinase recognition sequences is inserted between the rep gene and the promoter.

2. The system according to claim 1, which contains a vector for transferring a recombinase gene into a cell.

3. The system according to claim 2, wherein the vector for transferring a recombinase gene into a cell is an adenoviral vector.

4. The system according to claim 3, wherein the adenoviral vector containing the first nucleic acid, the adenoviral vector containing the second nucleic acid and the vector for transferring a recombinase gene into a cell are different each other.

5. The system according to claim 1, wherein the recombinase recognition sequences in the second nucleic acid are loxP nucleotide sequences, and the recombinase is Cre, a recombinase from Escherichia coli P1 phage.

6. A transformed cell into which a foreign gene is transferred using the system defined by claim 1.

7. A system for transferring a foreign gene into a cell, which system contains a combination of: a. an adenoviral vector containing a first nucleic acid which has a sequence in which ITRs from adeno-associated virus are positioned on both sides of a gene of interest to be transferred and a recombinase gene; and b. an adenoviral vector containing a second nucleic acid which has a rep gene from adeno-associated virus and a promoter for expressing the rep gene and in which a stuffer sequence put between two recombinase recognition sites is inserted between the rep gene and the promoter.

L17 ANSWER 3 OF 12 USPATFULL on STN
2005:274544 Method for amplifying nucleic acid sequence.
Mukai, Hiroyuki, Shiga, JAPAN
Sagawa, Hiroaki, Shiga, JAPAN
Uemori, Takashi, Shiga, JAPAN
Yamamoto, Junko, Shiga, JAPAN
Tomono, Jun, Shiga, JAPAN
Kobayashi, Eiji, Shiga, JAPAN
Enoki, Tatsuji, Shiga, JAPAN
Takeda, Osamu, Shiga, JAPAN
Miyake, Kazue, Kyoto, JAPAN
Sato, Yoshimi, Shiga, JAPAN
Moriyama, Mariko, Kyoto, JAPAN
Sawaragi, Haruhisa, Shiga, JAPAN
Hagiya, Michio, Shiga, JAPAN
Asada, **Kiyozo**, Shiga, JAPAN
Kato, Ikunoshin, Kyoto, JAPAN
TAKARA BIO INC., Shiga, JAPAN (non-U.S. corporation)
US 2005239100 A1 20051027
APPLICATION: US 2004-973919 A1 20041027 (10)
PRIORITY: JP 1999-76966 19990319
JP 1999-370035 19991227
JP 2000-251981 20000823
JP 2000-284419 20000919
JP 2000-288750 20000922
JP 2001-104191 20010403
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A convenient and effective method for amplifying a nucleic acid sequence characterized by effecting a DNA synthesis reaction in the presence of chimeric oligonucleotide primers; a method for supplying a large amount of DNA amplification fragments; an effective method for amplifying a nucleic acid sequence by combining the above method with another nucleic acid sequence amplification method; a method for detecting a nucleic acid sequence for detecting or quantitating a microorganism such as a virus, a bacterium, a fungus or a yeast; and a method for detecting a DNA amplification fragment obtained by the above method in situ.
CLM What is claimed is:
1. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product under conditions where specific annealing of the primer to the nucleic acid as the template, an extended strand synthesis reaction and a strand displacement reaction by the DNA polymerase, and a reaction of cleaving the extended strand by the endonuclease take place.

2. The method according to claim 1, wherein the reaction mixture is incubated isothermally.

3. The method according to claim 1, wherein the reaction mixture further contains another chimeric oligonucleotide primer having a sequence substantially homologous to the nucleotide sequence of the nucleic acid as the template.

4. The method according to claim 1, wherein the DNA polymerase is

I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

5. The method according to claim 1, wherein the endonuclease is an endoribonuclease.

6. The method according to claim 5, wherein the endoribonuclease is RNase H.

7. The method according to claim 6, wherein the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Thermotoga, an RNase H from a bacterium of genus Thermus, an RNase H from a bacterium of genus Pyrococcus, an RNase H from a bacterium of genus Archaeoglobus and an RNase H from a bacterium of genus Bacillus.

8. The method according to claim 1, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the RNase H as an endonuclease is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and an RNase H from a bacterium of genus Archaeoglobus.

9. The method according to claim 8, wherein the RNase H is type I RNase H from Escherichia coli, or type II RNase H from a bacterium of genus Pyrococcus or a bacterium of genus Archaeoglobus.

10. The method according to claim 1, wherein a DNA polymerase having an endonuclease activity is used.

11. The method according to claim 10, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the Bca DNA polymerase is used in the presence of a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

12. The method according to claim 11, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

13. The method according to claim 1, wherein the amplification reaction is conducted in the presence of a substance that inhibits the reverse transcription activity of the DNA polymerase.

14. The method according to claim 13, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

15. The method according to claim 1, wherein the chimeric oligonucleotide primer contains two or more successive ribonucleotide residues.

16. The method according to claim 1, wherein the chimeric oligonucleotide primer contains one or more modified ribonucleotide.

17. The method according to claim 16, wherein the chimeric oligonucleotide primer contains an (α-S) ribonucleotide in which the oxygen atom bound to the phosphorous atom at the α-position of the ribonucleotide is replaced by a sulfur atom.

18. The method according to claim 1, wherein a chimeric oligonucleotide primer represented by general formula below is used: 5'-dNa-Nb-dNc-3' General formula wherein a is an integer of 11 or more; b is an integer of 1 or more; c is 0 or an integer of 1 or more; dN is an deoxyribonucleotide and/or nucleotide analog; N is an unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-terminus by the action of the DNA polymerase does not take place.

19. The method according to claim 18, wherein c is 0.

20. The method according to claim 18, wherein the nucleotide analog is deoxyriboinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is (α-S) ribonucleotide.

21. The method according to claim 18, wherein the nucleic acid amplification reaction is conducted at a temperature suitable for the chimeric oligonucleotide primer as defined in claim 18.

22. The method according to claim 1, wherein the chimeric

selected from the group consisting of: a) a chimeric oligonucleotide primer for detecting enterohemorrhagic Escherichia coli having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 43 to 46, 136, 137, 140-142, 153-161, 173, 174, 202, 203, 219 and 220; b) a chimeric oligonucleotide primer for detecting a viroid having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 148, 149, 208, 209, 211, 212; c) a chimeric oligonucleotide primer for detecting Clostridium botulinum having a nucleotide sequence represented by SEQ ID NO: 205 or 206; d) a chimeric oligonucleotide primer for detecting papilloma virus having a nucleotide sequence represented by SEQ ID NO: 185 or 186; e) a chimeric oligonucleotide primer for detecting hepatitis C virus having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 190, 191, 227, 228, 289 and 290; f) a chimeric oligonucleotide primer for detecting Staphylococcus aureus having a nucleotide sequence represented by SEQ ID NO: 225 or 226; g) a chimeric oligonucleotide primer for detecting Mycobacterium tuberculosis having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 244, 245, 248 to 251, 283, 284; and h) a chimeric oligonucleotide primer for detecting Chlamydia having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 246 and 247.

23. The method according to claim 1, which is conducted in a buffer that contains a buffering component selected from the group consisting of Tricine, a phosphate, tris, Bicine and HEPES.

24. The method according claim 1, wherein the nucleic acid as the template is a single-stranded DNA or a double-stranded DNA.

25. The method according to claim 24, which is conducted after converting a double-stranded DNA as the template into single-stranded DNAs.

26. The method according to claim 24, wherein the nucleic acid as the template is a cDNA obtained from an RNA by a reverse transcription reaction.

27. The method according to claim 26, which is conducted after synthesizing a cDNA by a reverse transcription reaction using an RNA as a template.

28. The method according to claim 27, wherein a primer selected from the group consisting of an oligo-dT primer, a random primer and a specific primer is used as a primer for the reverse transcription reaction.

29. The method according to claim 27, wherein a chimeric oligonucleotide primer is used as a primer for the reverse transcription reaction.

30. The method according to claim 27, wherein a DNA polymerase having a reverse transcriptase activity is used as a reverse transcriptase.

31. The method according to claim 27, wherein the reverse transcription reaction and the nucleic acid amplification reaction are conducted using one DNA polymerase having a reverse transcriptase activity and a strand displacement activity.

32. The method according to claim 31, wherein the DNA polymerase is Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus or Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

33. The method according to claim 26, wherein the RNA as the template in the reverse transcription reaction is an RNA amplified by an additional nucleic acid amplification reaction.

34. The method according to claim 33, which is conducted after synthesizing an amplified RNA fragment by an additional nucleic acid amplification reaction using an RNA as a template.

35. The method according to claim 33, wherein the additional nucleic acid amplification reaction is selected from the group consisting of the transcription-based amplification system (TAS) method, the self-sustained sequence replication (3SR) method, the nucleic acid sequence-based amplification (NASBA) method, the transcription-mediated amplification (TMA) method and the Qβ replicase method.

36. The method according to claim 24, wherein the nucleic acid as the template is a DNA obtained by an additional nucleic acid amplification reaction.

37. The method according to claim 36, which is conducted after synthesizing an amplified DNA fragment by an additional nucleic acid

38. The method according to claim 36, wherein the additional nucleic acid amplification reaction is selected from the group consisting of the polymerase chain reaction (PCR) method, the ligase chain reaction (LCR) method and the strand displacement amplification (SDA) method.

39. The method according to claim 33, wherein a random primer or a degenerate primer is used for the additional nucleic acid amplification reaction.

40. The method according to claim 39, wherein the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

41. The method according to claim 36, wherein a random primer or a degenerate primer is used for the additional nucleic acid amplification reaction.

42. The method according to claim 41, wherein the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

43. The method according to claim 1, wherein the length of the region of the nucleic acid to be amplified is 200 bp or shorter.

44. The method according to claim 1, which comprises annealing the nucleic acid as the template to the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid in an annealing solution containing a substance that enhances the annealing of the nucleic acid to the primer.

45. The method according to claim 44, wherein the annealing solution contains spermidine and/or propylenediamine.

46. The method according to claim 44, wherein the annealing is conducted by incubating the annealing solution containing the nucleic acid as the template and the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid at 90° C. or above and then cooling the solution to a temperature at which the amplification reaction is conducted or below.

47. The method according to claim 1, wherein the nucleic acid amplification reaction is conducted in the presence of a deoxyribonucleotide triphosphate analog.

48. The method according to claim 47, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

49. The method according to claim 1, wherein a template switching reaction is effected.

50. The method according to claim 1, which further comprises a step of duplicating a DNA or an RNA containing a sequence to be amplified to prepare a nucleic acid as a template prior to step (a), wherein the duplicated nucleic acid is used in step (a) as a nucleic acid as a template.

51. A method for producing a nucleic acid in large quantities, characterized in that the method comprises: (a) amplifying a nucleic acid by the method defined by claim 1; and (b) collecting the nucleic acid amplified in step (a).

52. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises: (a) amplifying a target nucleic acid by the method defined by claim 1; and (b) detecting the target nucleic acid amplified in step (a).

53. The method according to claim 52, which comprises detecting the amplified nucleic acid using a probe for detection.

54. The method according to claim 53, wherein the probe for detection is a probe that has been labeled with a labeling substance.

55. The method according to claim 54, wherein the probe is an RNA probe labeled with two or more fluorescent substances positioned at a distance that results in a quenching state.

56. The method according to claim 53, wherein the probe is a probe which hybridizes to a region amplified using at least one chimeric oligonucleotide primer selected from the group consisting of: a) a chimeric oligonucleotide primer for detecting enterohemorrhagic

consisting of SEQ ID NOS: 43 to 46, 136, 137, 140-142, 153-161, 173, 174, 202, 203, 219 and 220; b) a chimeric oligonucleotide primer for detecting a viroid having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 148, 149, 208, 209, 211 and 212; c) a chimeric oligonucleotide primer for detecting Clostridium botulinum having a nucleotide sequence represented by SEQ ID NO: 205 or 206; d) a chimeric oligonucleotide primer for detecting papilloma virus having a nucleotide sequence represented by SEQ ID NO: 185 or 186; e) a chimeric oligonucleotide primer for detecting hepatitis C virus having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 190, 191, 227, 228, 289 and 290; f) a chimeric oligonucleotide primer for detecting Staphylococcus aureus having a nucleotide sequence represented by SEQ ID NO: 225 or 226; g) a chimeric oligonucleotide primer for detecting Mycobacterium tuberculosis having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 244, 245, 248 to 251, 283, 284; and h) a chimeric oligonucleotide primer for detecting Chlamydia having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 246 or 247.

57. A method for determining a nucleotide sequence of a nucleic acid, characterized in that the method comprises: (a) amplifying a nucleic acid by the method defined by claim 1; and (b) determining the nucleotide sequence of the nucleic acid amplified in step (a).

58. A method for preparing a single-stranded nucleic acid, the method comprising generating a single-stranded nucleic acid using the method defined by claim 1.

59. The method according to claim 58, wherein at least two primers at different concentrations are used.

60. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide; and (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

61. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide; (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b); (d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with an endonuclease at a site that contains the ribonucleotide; and (f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

62. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template and synthesize a double-stranded nucleic acid, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; and (c) reusing in step (b) the double-stranded nucleic acid obtained in step (b) as a template.

63. The method according to claim 62, wherein the DNA polymerase in step (a) is different from the DNA polymerase having a strand displacement activity in step (b).

64. A method for amplifying a nucleic acid using at least two primers, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; (c) reusing in step (b) the double-stranded nucleic acid obtained in step (b) as a template; (d) treating a displaced strand obtained in step (b) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (e) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; and (f) reusing in step (e) the double-stranded nucleic acid obtained in step (e) as a template.

65. The method according to claim 64, wherein the DNA polymerase in steps (a) and (d) is different from the DNA polymerase having a strand displacement activity in steps (b) and (f).

66. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; and (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the template and the primer-extended strand.

67. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a

nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; and (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other.

68. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; (d) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid to which the two primers are annealed obtained in step (c) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; and (e) reusing in step (d) the double-stranded nucleic acid to which the two primers are annealed obtained in step (d).

69. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; (d) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid to which the two primers are annealed obtained in step (c) to effect strand displacements and obtain a double-stranded nucleic acid

the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the template and the primer-extended strand obtained in step (d) with the endonuclease; and (f) extending a nucleic acid that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to synthesize a displaced strand.

70. The method according to claim 60, wherein step (b) and step (c) are sequentially repeated.

71. The method according to claim 60, wherein the DNA polymerase in step (a) is different from the DNA polymerase having a strand displacement activity in step (c).

72. The method according to claim 61, wherein one DNA polymerase having a strand displacement activity is used.

L17 ANSWER 4 OF 12 USPATFULL on STN
2005:144215 Method for amplifying nucleic acid sequence.
Mukai, Hiroyuki, Shiga, JAPAN
Sagawa, Hiroaki, Shiga, JAPAN
Uemori, Takashi, Shiga, JAPAN
Yamamoto, Junko, Shiga, JAPAN
Tomono, Jun, Shiga, JAPAN
Kobayashi, Eiji, Shiga, JAPAN
Enoki, Tatsuji, Shiga, JAPAN
Takeda, Osamu, Shiga, JAPAN
Miyake, Kazue, Kyoto, JAPAN
Sato, Yoshimi, Shiga, JAPAN
Moriyama, Mariko, Kyoto, JAPAN
Sawaragi, Haruhisa, Shiga, JAPAN
Hagiya, Michio, Shiga, JAPAN
Asada, Kiyozo, Shiga, JAPAN
Kato, Ikunoshin, Kyoto, JAPAN
TAKARA BIO NIC., Shiga, JAPAN (non-U.S. corporation)
US 2005123950 A1 20050609
APPLICATION: US 2004-929759 A1 20040831 (10)
PRIORITY: JP 1999-76966 19990319
JP 1999-370035 19991227
JP 2000-251981 20000823
JP 2000-284419 20000919
JP 2000-288750 20000922
JP 2001-104191 20010403
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A convenient and effective method for amplifying a nucleic acid sequence characterized by effecting a DNA synthesis reaction in the presence of chimeric oligonucleotide primers; a method for supplying a large amount of DNA amplification fragments; an effective method for amplifying a nucleic acid sequence by combining the above method with another nucleic acid sequence amplification method; a method for detecting a nucleic acid sequence for detecting or quantitating a microorganism such as a virus, a bacterium, a fungus or a yeast; and a method for detecting a DNA amplification fragment obtained by the above method in situ.

CLM What is claimed is:

1. A kit used for a method for amplifying a nucleic acid, wherein said kit is in a packaged form and contains instructions that direct the use of a DNA polymerase and an endonuclease and said method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

2. The kit according to claim 1, which further contains at least one selected from the group consisting of: (a) a DNA polymerase having a strand displacement activity; (b) an endonuclease; and (c) a buffer.

3. A kit used for the method for detecting a target nucleic acid, wherein said kit is in a packaged form and contains instructions that direct the use of a DNA polymerase and an endonuclease in a strand displacement reaction and wherein said method comprises: (a) amplifying a target nucleic acid by preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA

and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer and incubating the reaction mixture for a sufficient time to generate a reaction product; and (b) detecting the target nucleic acid amplified in step (a).

4. The kit according to claim 3, which contains at least one selected from the group consisting of: (a) a DNA polymerase having a strand displacement activity; (b) an endonuclease; and (c) a buffer.

5. A composition for amplifying a nucleic acid used for a method for amplifying a nucleic acid, which contains: an endonuclease; and a DNA polymerase having a strand displacement activity, wherein said method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

6. The composition according to claim 5, which further contains at least one primer that is substantially complementary to a nucleotide sequence of a nucleic acid as a template, wherein the primer is a chimeric oligonucleotide primer that contains a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer.

7. The composition according to claim 5, which contains a buffering component suitable for a nucleic acid amplification reaction.

8. A composition for amplifying a nucleic acid which contains: (a) at least one primer that is substantially complementary to a nucleotide sequence of a nucleic acid as a template, wherein the primer is a chimeric oligonucleotide primer that contains a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) an endonuclease; and (c) a DNA polymerase having a strand displacement activity.

9. A composition for amplifying a nucleic acid which contains: (a) at least two primers that are substantially complementary to nucleotide sequences of respective strands of a double-stranded nucleic acid as a template, wherein each primer is a chimeric oligonucleotide primer that contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) an endonuclease; and (c) a DNA polymerase having a strand displacement activity.

10. A composition for amplifying a nucleic acid obtained by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer.

11. A composition for amplifying a nucleic acid obtained by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least two primers and an endonuclease, wherein each primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of each strand of the double-stranded nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer.

12. A chimeric oligonucleotide primer used for a method for amplifying a

nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein said method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

13. A chimeric oligonucleotide primer used for a method for detecting a target nucleic acid, which is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein said method comprises: (a) amplifying a target nucleic acid by preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer and incubating the reaction mixture for a sufficient time to generate a reaction product; and (b) detecting the target nucleic acid amplified in step (a).

14. The chimeric oligonucleotide primer according to claim 13, which is a chimeric oligonucleotide primer for detecting a pathogenic microorganism or a disease-related gene.

15. The chimeric oligonucleotide primer according to claim 14, wherein the pathogenic microorganism is enterohemorrhagic Escherichia coli, Clostridium botulinum, Staphylococcus aureus, Mycobacterium tuberculosis, Chlamydia, papilloma virus, hepatitis C virus or a viroid.

16. A method for producing a material having an immobilized nucleic acid in which the nucleic acid is arrayed in a predefined region, characterized in that the method comprises: (a) amplifying a nucleic acid to be immobilized by preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer and incubating the reaction mixture for a sufficient time to generate a reaction product; and (b) arraying and immobilizing the nucleic acid amplified in step (a) in a predefined region on a substrate.

17. The method according to claim 16, wherein a single-stranded nucleic acid substantially free of a complementary strand thereto is amplified, and arrayed and immobilized in the predefined region on the substrate.

18. A material having an immobilized nucleic acid in which the nucleic acid is arrayed in a predefined region produced by the method defined by claim 16.

19. The material according to claim 18, wherein a single-stranded nucleic acid is arrayed and immobilized in the predefined region.

20. The material according to claim 19, in which a single-stranded nucleic acid substantially free of a complementary strand thereto is arrayed and immobilized in the predefined region.

21. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises: (a) preparing a nucleic acid sample suspected to contain a target nucleic acid from a sample; (b) contacting the nucleic acid sample with the material defined by

sample that hybridizes with the nucleic acid on the material.

22. A product of a reagent for amplifying a nucleic acid consisting of a packing material and a reagent for amplifying a nucleic acid enclosed in the packing material, wherein the reagent for amplifying a nucleic acid contains a DNA polymerase having a strand displacement activity and/or an RNase H, and description that the reagent for amplifying a nucleic acid can be used for nucleic acid amplification under isothermal conditions is indicated in a label stuck to the packaging material or instructions attached to the packaging material.

23. A product of a reagent for amplifying a nucleic acid consisting of a packing material and a reagent for amplifying a nucleic acid enclosed in the packing material, wherein the reagent for amplifying a nucleic acid contains a DNA polymerase having a strand displacement activity and/or an endonuclease, and description that the reagent for amplifying a nucleic acid can be used for nucleic acid amplification under isothermal conditions is indicated in a label stuck to the packaging material or instructions attached to the packaging material.

L17 ANSWER 5 OF 12 USPATFULL on STN
2004:223723 Gene transfer methods.
Ueno, Mitsuhiro, Kusatsu, JAPAN
Yoshioka, Hirofumi, Kusatsu, JAPAN
Konishi, Haruko, Kyoto, JAPAN
Hashino, Kimikazu, Takatsuki, JAPAN
Morishita, Mio, Otsu, JAPAN
Chono, Hideto, Moriyama, JAPAN
Miyamura, Tsuyoshi, Kusatsu, JAPAN
Sano, Mutsumi, Otsu, JAPAN
Asada, Kiyozo, Shiga, JAPAN
Fujinaga, Kei, Otsu, JAPAN
Kato, Ikunoshin, Uji, JAPAN
Takara Shuzo Co., Ltd., Kyoto, JAPAN (non-U.S. corporation)
US 6787359 B1 20040907
WO 2000001836 20000113
APPLICATION: US 2001-743354 20010109 (9)
WO 1999-JP3403 19990625
PRIORITY: JP 1998-186240 19980701
JP 1999-56915 19990304
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Improved methods for transferring a gene into target cells by using a **retrovirus**, wherein the gene transfer efficiency is improved and the target cells are efficiently transformed by binding the **retrovirus** to a functional substance which is immobilized on as carrier and having an activity of binding to **retroviruses** followed by washing; using an antibody capable of specifically recognizing cells, laminin or mannose-rich type sugar chain as a substance having an activity of binding to the target cells; pre-treating the target cells so as to inactivate transferrin receptor, or introducing a new functional group into the functional substance.
CLM What is claimed is:
1. A method for transferring a gene into target cells using a **retrovirus**, comprising: (1) contacting a solution containing a **retrovirus** with a functional substance that binds to the **retrovirus** and is immobilized on a substrate for 3 hours or longer; (2) washing the substrate to which the **retrovirus** is bound; and (3) contacting and incubating the substance to which the **retrovirus** is bound with target cells.

2. The method according to claim 1, wherein step (1) is carried out by precipitating the **retrovirus** by centrifugal force onto the functional substance that binds to the **retrovirus** and being immobilized on the substrate.

3. The method according to claim 1, wherein the functional substance that binds to the **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

4. The method according to claim 1, wherein the functional substance that binds to the **retrovirus** binds to target cells.

5. The method according to 1, wherein a substrate is used on which the functional substance that binds to the **retrovirus** and another functional substance that binds to the target cells are immobilized.

6. The method according to claim 1, wherein a vessel for cell culture or a particulate substrate is used as the substrate.

retrovirus is a culture supernatant of **retrovirus**-producer cells obtained in the presence of a substance that enhances **retrovirus** production.

8. A method for transferring a gene into target cells using a **retrovirus**, comprising: (1) contacting a solution containing a **retrovirus** with a functional substance that binds to the **retrovirus** and being immobilized on a substrate; (2) washing the substrate to which the **retrovirus** is bound; and (3) contacting and incubating the substrate to which the **retrovirus** is bound with target cells, wherein the frequency of contact between the **retrovirus** and the functional substance having an activity of binding to the **retrovirus** is physically increased in step (1).

9. The method according to claim 5, wherein the functional substance that binds to the target cells is selected from the group consisting of cell-adhesive proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

10. The method according to claim 7, wherein the solution containing the **retrovirus** is a culture supernatant obtained in the presence of sodium butyrate.

11. The method according to claim 8, wherein step (1) is carried out by precipitating the **retrovirus** by centrifugal force onto the functional substance having an activity of binding to the **retrovirus** and being immobilized on the substrate.

12. The method according to claim 8, wherein the functional substance that binds to the **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

13. The method according to claim 8, wherein the functional substance that binds to the **retrovirus** binds to target cells.

14. The method according to claim 8, wherein a substrate is used on which the functional substance that binds to the **retrovirus** and another functional substance that binds to the target cells are immobilized.

15. The method according to claim 8, wherein a vessel for cell culture or a particulate substrate is used as the substrate.

16. The method according to claim 8, wherein the solution containing the **retrovirus** is a culture supernatant of **retrovirus**-producer cells obtained in the presence of a substance that enhances **retrovirus** production.

17. A method for transferring a gene into target cells using a **retrovirus**, characterized in that the method comprises infecting target cells with a **retrovirus** in the presence of two functional substances: (1) a functional substance having an activity of binding to the **retrovirus**; and (2) an antibody which specifically binds to a CD antigen expressed on the target cells.

18. The method according to claim 17, wherein the functional substance that binds to the **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

19. The method according to claim 17, wherein at least one of the two functional substances is immobilized on a substrate.

20. A method for transferring a gene into target cells using a **retrovirus**, characterized in that the method comprises infecting target cells with a **retrovirus** in the presence of two functional substances: (1) a functional substance that binds to the **retrovirus**; and (2) a sugar chain derived from laminin or a high mannose type sugar chain.

21. The method according to claim 18, wherein the functional substance that binds to the **retrovirus** has an activity of binding to target cells.

22. The method according to claim 19, wherein a vessel for cell culture or a particulate substrate is used as the substrate.

23. The method according to claim 20, wherein the functional substance that binds to the **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

functional substances is immobilized on a substrate.

25. The method according to claim 20, wherein the functional substance that binds to the **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

26. The method according to claim 20, wherein at least one of the two functional substances is immobilized on a substrate.

L17 ANSWER 6 OF 12 USPATFULL on STN
2004:76669 Gene transfer methods.
Ueno, Mitsuhiro, Kasatsu-shi, JAPAN
Yoshioka, Hirofumi, Kasatsu-shi, JAPAN
Konishi, Haruko, Kyoto-shi, JAPAN
Hashino, Kimikazu, Osaka, JAPAN
Morishita, Mio, Otsu-shi, JAPAN
Chono, Hideto, Moriyama-shi, JAPAN
Miyamura, Tsuyoshi, Kusatsu-shi, JAPAN
Sano, Mutsumi, Otsu-shi, JAPAN
Asada, **Kiyozo**, Koka-gun, JAPAN
Fujinaga, Kei, Otsu-shi, JAPAN
Kato, Ikunoshin, Uji-shi, JAPAN
Takara Shuzo Co., Ltd., Kyoto-shi, JAPAN (non-U.S. corporation)
US 2004058447 A1 20040325
APPLICATION: US 2003-657076 A1 20030909 (10)
PRIORITY: JP 1998-186240 19980701
JP 1999-56915 19990304
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Improved methods for transferring a gene into target cells by using a **retrovirus**, wherein the gene transfer efficiency is improved and the target cells are efficiently transformed by binding the **retrovirus** to a functional substance which is immobilized on as carrier and having an activity of binding to **retroviruses** followed by washing; using an antibody capable of specifically recognizing cells, laminin or mannose-rich type sugar chain as a substance having an activity of binding to the target cells; pre-treating the target cells so as to inactivate transferrin receptor, or introducing a new functional group into the functional substance.

CLM What is claimed is:

1. A method for transferring a gene into target cells using a **retrovirus**, comprising culturing target cells in a medium that contains Fe at a lowered concentration before the target cells are contacted with the **retrovirus**.

2. The method according to claim 1, wherein target cells are cultured in a medium that contains deferoxamine before the target cells are contacted with the **retrovirus**.

3. A method for increasing activity of binding a peptide or a protein to a **retrovirus**, comprising chemically modifying a peptide or a protein.

4. The method according to claim 3, which comprises treating the peptide or the protein with a water soluble carbodiimide.

5. The method according to claim 4, which comprises treating the peptide or the protein with a water-soluble carbodiimide and a diamino compound.

6. The method according to claim 3, wherein the peptide or the protein is selected from the group consisting of fibronectin, fibroblast growth factor and collagen type V, as well as fragments thereof and substances having an equivalent activity of binding to the **retrovirus** thereto.

7. A functional substance having an activity of binding a **retrovirus** which contains a peptide or a protein treated by the method according to claim 3.

8. A method for transferring a gene into target cells, comprising infecting target cells with a **retrovirus** in the presence of the functional substance having an activity of binding a **retrovirus** according to claim 7.

9. The method according to claim 4, wherein the peptide or the protein is selected from the group consisting of fibronectin, fibroblast growth factor and collagen type V, as well as fragments thereof and substances having an equivalent activity of binding to the **retrovirus** thereto.

10. The method according to claim 5, wherein the peptide or the protein is selected from the group consisting of fibronectin, fibroblast growth factor and collagen type V, as well as fragments thereof and substances

11. A functional substance having an activity of binding a **retrovirus** which contains a peptide or a protein treated by the method according to claim 10.

12. A method for transferring a gene into target cells, comprising infecting target cells with a **retrovirus** in the presence of the functional substance having an activity of binding a **retrovirus** according to claim 11.

13. A method for gene therapy, comprising, (1) contacting a solution containing a **retrovirus** with a functional substance that binds to the **retrovirus** and being immobilized on a substrate for 3 hours or longer; (2) washing the substrate to which the **retrovirus** is bound; (3) contacting and incubating the substrate to which the **retrovirus** is bound with target cells collected from a donor; and (4) transplanting the cell obtained tin step (3) into a recipient.

14. A method for gene therapy, comprising, (1) contacting a solution containing a **retrovirus** with a functional substance that binds to the **retrovirus** and being immobilized on a substrate; (2) washing the substrate to which the **retrovirus** is bound; (3) contacting and incubating the substrate to which the **retrovirus** is bound with target cells collected from a donor; and (4) transplanting the cell obtained in step (3) into a recipient, wherein the frequency of contact between the **retrovirus** and the functional substance that binds to the **retrovirus** is physically increased in step (1).

15. The method according to claim 14, wherein step (1) is carried out by precipitating the **retrovirus** by centrifugal force onto the functional substance that binds to the **retrovirus** and being immobilized on a substrate.

16. The method according to claim 14, wherein the functional substance that binds to the **retrovirus** has an activity of binding to the target cells.

17. The method according to claim 14, wherein the substrate on which the functional substance that binds to the **retrovirus** and another functional substance that binds to the target cells are immobilized is used.

18. The method according to claim 17, wherein a vessel for cell culture or a particulate substrate is used as the substrate.

19. The method according to claim 14, wherein the solution containing the **retrovirus** is a culture supernatant of **retrovirus** producer cells obtained in the presence of a substance that enhances **retrovirus** production.

20. The method according to claim 19, wherein the solution containing the **retrovirus** is a culture supernatant obtained in the presence of sodium butylate.

21. A method for gene therapy, comprising, (1) infecting target cells collected from a donor with a **retrovirus** in the presence of two functional substances; a) a functional substance that binds to the **retrovirus**; and b) an antibody which specifically binds to a CD antigen expressed on the target cells; and (2) transplanting the cell obtained in step (1) into a recipient.

22. A method for gene therapy, comprising, (1) infecting target cells collected from a donor with a **retrovirus** in the presence of two functional substances; a) a functional substance that binds to the **retrovirus**; and b) a sugar chain derived from laminin or a high mannose type sugar chain; and (2) transplanting the cell obtained in step (1) into a recipient.

23. The method according to claim 22, wherein the functional substance that binds to the **retrovirus** has an activity of binding to the target cells.

24. The method according to claim 22, wherein at least one functional substance is immobilized on a substrate.

25. The method according to claim 24, wherein a vessel for cell culture or a particulate substrate is used as the substrate.

26. The method according to claim 22, wherein the recipient is the donor itself.

27. The method according to claim 22, wherein the target cells are

28. The method according to claim 22, wherein the functional substance which binds to **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

29. The method according to claim 13, wherein the functional substance that binds to the **retrovirus** has an activity of binding to the target cells.

30. The method according to claim 13, wherein the substrate on which the functional substance that binds to the **retrovirus** and another functional substance that binds to the target cells are immobilized is used.

31. The method according to claim 30, wherein a vessel for cell culture or a particulate substrate is used as the substrate.

32. The method according to claim 13, wherein the solution containing the **retrovirus** is a culture supernatant of **retrovirus** producer cells obtained in the presence of a substance that enhances **retrovirus** production.

33. The method according to claim 32, wherein the solution containing the **retrovirus** is a culture supernatant obtained in the presence of sodium butylate.

34. The method according to claim 21, wherein the functional substance that binds to the **retrovirus** has an activity of binding to the target cells.

35. The method according to claim 21, wherein at least one functional substance is immobilized on a substrate.

36. The method according to claim 35, wherein a vessel for cell culture or a particulate substrate is used as the substrate.

37. The method according to claim 21, wherein the recipient is the donor itself.

38. The method according to claim 21, wherein the target cells are hematopoietic stem cells.

39. The method according to claim 21, wherein the functional substance which binds to **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

40. The method according to claim 13, wherein the recipient is the donor itself.

41. The method according to claim 13, wherein the target cells are hematopoietic stem cells.

42. The method according to claim 13, wherein the functional substance which binds to **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

43. The method according to claim 14, wherein the recipient is the donor itself.

44. The method according to claim 14, wherein the target cells are hematopoietic stem cells.

45. The method according to claim 14, wherein the functional substance which binds to **retrovirus** is selected from the group consisting of fibronectin, fibroblast growth factor, collagen type V, polylysine and DEAE-dextran, as well as fragments thereof.

L17 ANSWER 7 OF 12 USPATFULL on STN
2003:127224 Method for gene transfer into target cells with **retrovirus**.
Asada, Kiyozo, Koka-gun, JAPAN
Uemori, Takashi, Otsu-shi, JAPAN
Ueno, Takashi, Kusatsu-shi, JAPAN
Koyama, Nobuto, Uji-shi, JAPAN
Hashino, Kimikazu, Osaka, JAPAN
Kato, Ikunoshin, Uji-shi, JAPAN
US 2003087437 A1 20030508
APPLICATION: US 2001-775964 A1 20010202 (9)
PRIORITY: JP 1995-294382 19951113

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**. The transduction is effected by infecting target cells with a **retrovirus** in the presence of a mixture of a functional material having a **retrovirus** binding domain, and a second functional material having target cell binding domain. The target cells may be selected from the group of unipotential hematopoietic progenitor cells and erythrocyte precursor, specifically pluripotent stem cells or embryopalstic stem cells.

CLM What is claimed is:

1. In a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**, wherein the improvement comprises carrying out the transduction by infecting the target cells with the **retrovirus** in the presence of a mixture of an effective amount of a functional material having **retrovirus** binding domain, and an effective amount of another functional material having target cell binding domain.

2. A method according to claim 1, wherein the functional material having **retrovirus** binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

3. A method according to claim 1, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

4. A method according to claim 3, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

5. A method according to claim 4, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

6. A method according to claim 5, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

7. A method according to claim 4, wherein the ligand is erythropoietin.

8. A method according to any one of claims 1 to 7, wherein the functional materials are immobilized.

9. A culture medium for target cells to be used for gene transfer into the target cells with a **retrovirus** which comprises a mixture of an effective amount of a functional material having **retrovirus** binding domain, and an effective amount of another functional material having target cell binding domain.

10. A culture medium according to claim 9, wherein the functional material having **retrovirus** binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

11. A culture medium according to claim 9, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

12. A culture medium according to claim 11, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

13. A culture medium according to claim 12, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

14. A culture medium according to claim 13, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

15. A culture medium according to claim 12, wherein the ligand is erythropoietin.

16. A culture medium according to any one of claims 9 to 15, wherein the functional materials are immobilized.

17. A method for localization of a **retrovirus** which comprises incubating a culture medium containing the **retrovirus** contacted with a mixture of an effective amount of a functional material having **retrovirus** binding domain, and an effective amount of another functional material having target cell binding domain.

functional material having **retrovirus** binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

19. A method for localization according to claim 17, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

20. A method for localization according to claim 19, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

21. A method for localization according to claim 20, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

22. A method for localization according to claim 21, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

23. A method for localization according to claim 20, wherein the ligand is erythropoietin.

24. A method for localization according to any one of claims 17 to 23, wherein the functional materials are immobilized.

25. A kit for carrying out **retrovirus**-mediated gene transfer into target cells, which comprises: (a) an effective amount of a functional material having **retrovirus** binding domain and/or an effective amount of another functional material having target cell binding domain; (b) an artificial substrate for incubating the **retrovirus** and the target cells; and (c) a target cell growth factor for pre-stimulating the target cells.

26. A kit according to claim 25, wherein the functional material having **retrovirus** binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

27. A kit according to claim 25, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

28. A kit according to claim 27, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

29. A kit according to claim 27, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

30. A kit according to claim 29, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

31. A kit according to claim 28, wherein the ligand is erythropoietin.

32. A kit according to any one of claims 25 to 31, wherein the functional materials are immobilized.

33. A method for localization of a **retrovirus** comprising incubating a culture medium containing the **retrovirus** contacted with an effective amount of a functional material having a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine.

34. A method for localization according to claim 33, wherein the functional material is immobilized.

35. In a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**, wherein the improvement comprises carrying out the transduction by infecting the target cells with the **retrovirus** in the presence of an effective amount of a functional material having a target cell binding domain, and a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

36. A method according to claim 35, wherein the target cell binding domain is a ligand which specifically binds to the target cells.

37. A method according to claim 36, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

a cell binding domain polypeptide of fibronectin.

39. A method according to claim 38, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

40. A method according to claim 37, wherein the ligand is erythropoietin.

41. A method according to claim 35, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

42. A method according to claim 35, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

43. A method according to claim 35, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalent of the fragment.

44. A method according to claim 43, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

45. A method according to claim 35, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

46. A method according to any one of claims 35 to 45, wherein the functional material is immobilized.

47. A method according to any one of claims 35 to 45, wherein the functional material is used without immobilization.

48. A culture medium for target cells to be used for gene transfer into the target cells with a **retrovirus** which comprises an effective amount of a functional material having a target cell binding domain, and a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

49. A culture medium according to claim 48, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

50. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

51. A culture medium according to claim 48, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalents of the fragment.

52. A culture medium according to claim 48, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

53. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

54. A culture medium according to any one of claims 48 to 53, wherein the functional material is immobilized.

55. A method for localization of a **retrovirus** which comprises incubating a culture medium containing the **retrovirus** contacted with an effective amount of a functional material having a target cell binding domain, and a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

56. A method for localization according to claim 55, wherein the fibroblast growth factor is selected from a fibroblast growth factor

equivalents of the factor and polypeptides containing the factor or functional equivalents of the factor.

57. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

58. A method for localization according to claim 55, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalent of the fragment.

59. A method for localization according to claim 58, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

60. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

61. A method for localization according to any one of claims 50 to 60, wherein the functional material is immobilized.

62. A kit for carrying out **retrovirus**-mediated gene transfer into target cells, which comprises: (a) an effective amount of a functional material having a target cell binding domain, and a **retrovirus** binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule; (b) an artificial substrate for incubating the **retrovirus** and the target cells; and (c) a target cell growth factor for pre-stimulating the target cells.

63. A kit according to claim 62, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

64. A kit according to claim 62, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

65. A kit according to claim 62, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalents of the fragment.

66. A kit according to claim 65, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

67. A kit according to claim 62, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

68. A kit according to any one of claims 62 to 67, wherein the functional material is immobilized.

69. A method according to claim 8 or 46, wherein the functional materials are immobilized on beads.

70. A culture medium according to claim 16 or 54, the functional materials are immobilized on beads.

71. A method for immobilization according to claim 24, 34 or 61, wherein the functional materials are immobilized on beads.

72. A kit according to claim 32 or 68, wherein the functional materials are immobilized on beads.

73. In a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**, wherein the improvement comprises infecting the target cells with the **retrovirus** in the presence of an effective amount of a functional material selected from the group consisting of substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof which is immobilized on beads.

74. In a method for increasing the efficiency of gene transfer into target cells with a **retrovirus**, wherein the improvement comprises

effective amount of a functional material selected from the group consisting of substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof which is not immobilized.

75. A method according to any one of claims 1 to 8, 35 to 47, 69, 73 and 74, wherein the target cells are cells selected from stem cells, hematopoietic cells, non-adherent low density mononuclear cells, adherent cells, bone marrow cells, hematopoietic stem cells, peripheral blood stem cells, umbilical blood cells, fetal hematopoietic stem sells, embryoplastic stem cells, embryonic cells, primordial germ cell, oocyte, oogonia, ova, spermatocyte, sperm, CD 34+cells, C-kit+cells, multipotential hematopoietic progenitor cells, unipotential hematopoietic progenitor cells, erythrocyte precursor cells, lymphocytic precursor cells, mature blood cells, lymphocytes, B cells, T cells, fibroblasts, neuroblasts, nerve cells, endothelial cells, angio-endothelial cells, hepatic cells, myoblasts, skeletal muscle cells, smooth muscle cells, cancer cells, myeloma cells and leukemia cells.

76. A method according to any one of claims 1 to 8, 17 to 24, 33 to 47, 55 to 61, 69, 71 and 73 to 75, wherein the **retrovirus** includes an exogenous gene.

77. A method according to claim 76, wherein the **retrovirus** is a recombinant **retroviral** vector.

78. A method according to claim 76, wherein the **retrovirus** is a replication deficient recombinant **retroviral** vector.

79. Transformant cells obtained by a method according to any one of claims 1 to 8, 35 to 47, 69 and 73 to 78.

80. A method for cellular grafting comprising grafting the transformant cells obtained by a method according to claim 79 into a vertebrate animal.

81. A polypeptide represented by SEQ. ID 13 of the Sequence Listing.

82. A gene encoding the polypeptide according to claim 81.

83. A gene according to claim 82 which is represented by SEQ. ID No. 17 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a **retrovirus**.

84. A polypeptide represented by SEQ. ID No. 30 of the Sequence Listing or functional equivalents thereof.

85. A gene encoding the polypeptide according to claim 84.

86. A gene according to claim 85 which is represented by SEQ. ID No. 33 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a **retrovirus**.

87. A polypeptide represented by SEQ. ID No. 5 of the Sequence Listing or functional equivalents thereof.

88. A gene encoding the polypeptide according to claim 87.

89. A gene according to claim 88 which is represented by SEQ. ID No. 26 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a **retrovirus**.

L17 ANSWER 8 OF 12 USPATFULL on STN
2003:106170 Method for amplifying nucleic acid sequence.
Mukai, Hiroyuki, Shiga, JAPAN
Sagawa, Hiroaki, Shiga, JAPAN
Uemori, Takashi, Shiga, JAPAN
Yamamoto, Junko, Shiga, JAPAN
Tomono, Jun, Shiga, JAPAN
Kobayashi, Eiji, Shiga, JAPAN
Enoki, Tatsuji, Shiga, JAPAN
Takeda, Osamu, Shiga, JAPAN
Miyake, Kazue, Kyoto, JAPAN
Sato, Yoshimi, Shiga, JAPAN
Moriyama, Mariko, Kyoto, JAPAN
Sawaragi, Haruhisa, Shiga, JAPAN
Hagiya, Michio, Shiga, JAPAN
Asada, Kiyozo, Shiga, JAPAN

Takara Shuzo Co., Ltd, Kyoto-shi, JAPAN (non-U.S. corporation)
US 2003073081 A1 20030417
APPLICATION: US 2001-935338 A1 20010823 (9)
PRIORITY: JP 1999-76966 19990319
JP 1999-370035 19991227
JP 2000-251981 20000823
JP 2000-284419 20000919
JP 2000-288750 20000922
JP 2001-104191 20010403
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A convenient and effective method for amplifying a nucleic acid sequence characterized by effecting a DNA synthesis reaction in the presence of chimeric oligonucleotide primers; a method for supplying a large amount of DNA amplification fragments; an effective method for amplifying a nucleic acid sequence by combining the above method with another nucleic acid sequence amplification method; a method for detecting a nucleic acid sequence for detecting or quantitating a microorganism such as a virus, a bacterium, a fungus or a yeast; and a method for detecting a DNA amplification fragment obtained by the above method in situ.

CLM What is claimed is:

1. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide; and (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

2. The method according to claim 1, wherein step (b) and step (c) are sequentially repeated.

3. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide; (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b); (d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with the endonuclease at a site that contains the ribonucleotide; and (f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

4. The method according to any one of claims 1 to 3, wherein the respective steps are conducted isothermally.

5. The method according to any one of claims 1 to 4, wherein one DNA

6. The method according to any one of claims 1 to 5, wherein the DNA polymerase is selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

7. The method according to any one of claims 1 to 6, wherein the endonuclease is an endoribonuclease.

8. The method according to claim 7, wherein the endoribonuclease is RNase H.

9. The method according to any one of claims 1 to 8, wherein the chimeric oligonucleotide primer contains two or more successive ribonucleotide residues.

10. The method according to any one of claims 1 to 9, wherein the chimeric oligonucleotide primer contains one or more modified ribonucleotide.

11. The method according to claim 10, wherein the chimeric oligonucleotide primer contains an (α-S) ribonucleotide in which the oxygen atom bound to the phosphorous atom at the α-position of the ribonucleotide is replaced by a sulfur atom.

12. The method according to any one of claims 1 to 11, which is conducted in a buffer that contains a buffering component selected from the group consisting of Tricine, a phosphate and tris.

13. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with the endonuclease; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

14. The method according to claim 13, wherein the reaction mixture is incubated isothermally.

15. The method according to claim 13 or 14, wherein the reaction mixture further contains a chimeric oligonucleotide primer having a sequence substantially homologous to the nucleotide sequence of the nucleic acid as the template.

16. The method according to any one of claims 13 to 15, wherein the DNA polymerase is selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

17. The method according to any one of claims 13 to 16, wherein the endonuclease is an endoribonuclease.

18. The method according to claim 17, wherein the endoribonuclease is RNase H.

19. The method according to any one of claims 13 to 18, wherein the chimeric oligonucleotide primer contains two or more successive ribonucleotide residues.

20. The method according to any one of claims 13 to 19, wherein the chimeric oligonucleotide primer contains one or more modified ribonucleotide.

21. The method according to claim 20, wherein the chimeric oligonucleotide primer contains an (α-S) ribonucleotide in which the oxygen atom bound to the phosphorous atom at the α-position of the ribonucleotide is replaced by a sulfur atom.

22. The method according to any one of claims 13 to 21, which is conducted in a buffer that contains a buffering component selected from the group consisting of Tricine, a phosphate and tris.

23. The method according any one of claims 1 to 22, wherein the nucleic

24. The method according to claim 23, which is conducted after converting a double-stranded DNA as the template into single-stranded DNAs.

25. The method according to claim 23 or 24, wherein the nucleic acid as the template is a cDNA obtained from an RNA by a reverse transcription reaction.

26. The method according to claim 25, which is conducted after synthesizing a cDNA by a reverse transcription reaction using an RNA as a template.

27. The method according to claim 26, wherein a primer selected from the group consisting of an oligo-dT primer, a random primer and a specific primer is used as a primer for the reverse transcription reaction.

28. The method according to claim 26 or 27, wherein a chimeric oligonucleotide primer is used as a primer for the reverse transcription reaction.

29. The method according to any one of claims 26 to 28, wherein a DNA polymerase having a reverse transcriptase activity is used as a reverse transcriptase.

30. The method according to any one of claims 26 to 29, wherein the reverse transcription reaction and the synthesis of the extended strand that is complementary to the template are conducted using one DNA polymerase having a reverse transcriptase activity and a strand displacement activity.

31. The method according to claim 28, wherein the DNA polymerase is Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus or Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

32. The method according to any one of claims 25 to 31, wherein the RNA as the template in the reverse transcription reaction is an RNA amplified by a nucleic acid amplification reaction.

33. The method according to claim 32, which is conducted after synthesizing an amplified RNA fragment by a nucleic acid amplification reaction using an RNA as a template.

34. The method according to claim 32 or 33, wherein the nucleic acid amplification reaction is selected from the group consisting of the transcription-based amplification system (TAS) method, the self-sustained sequence replication (3SR) method, the nucleic acid sequence-based amplification (NASBA) method, the transcription-mediated amplification (TMA) method and the Qβ replicase method.

35. The method according to claim 23 or 24, wherein the nucleic acid as the template is a DNA obtained by a nucleic acid amplification reaction.

36. The method according to claim 35, which is conducted after synthesizing an amplified DNA fragment by a nucleic acid amplification reaction using an DNA as a template.

37. The method according to claim 36, wherein the nucleic acid amplification reaction is selected from the group consisting of the polymerase chain reaction (PCR) method, the ligase chain reaction (LCR) method and the strand displacement amplification (SDA) method.

38. The method according to any one of claims 32 to 37, wherein a random primer or a degenerate primer is used for the nucleic acid amplification reaction.

39. The method according to claim 38, wherein the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

40. A chimeric oligonucleotide primer used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39, which contains a deoxyribonucleotide and a ribonucleotide, and has a structure in which the ribonucleotide is position at the 3'-terminus or on the 3'-terminal side of the primer.

41. The chimeric oligonucleotide primer according to claim 40, which contains two or more successive ribonucleotide residues.

42. The chimeric oligonucleotide primer according to claim 40 or 41,

43. The chimeric oligonucleotide primer according to claim 42, which contains an (α-S) ribonucleotide in which the oxygen atom bound to the phosphorous atom at the α-position of the ribonucleotide triphosphate is replaced by a sulfur atom as the modified ribonucleotide.

44. A DNA polymerase having a strand displacement activity used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39.

45. An endonuclease used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39.

46. A kit used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39, which is in a packaged form and contains instructions that direct the use of a DNA polymerase and an endonuclease in a strand displacement reaction.

47. The kit according to claim 46, which contains a DNA polymerase and/or an endonuclease.

48. The kit according to claim 47, which is in a package form and contains: (a) a DNA polymerase having a strand displacement activity; (b) an endonuclease; and (c) a buffer for a strand displacement reaction.

49. A kit used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39, which contains the DNA polymerase having the strand displacement activity according to claim 44 and/or the endonuclease according to claim 45.

50. The kit according to any one of claims 47 to 49, which contains a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax as the DNA polymerase.

51. The kit according to any one claims 47 to 49, which contains RNase H as the endonuclease.

52. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises: (a) amplifying a target nucleic acid by the method for amplifying a nucleotide sequence according to claims 1 to 39; and (b) detecting the target nucleic acid amplified in step (a).

53. The method according to claim 52, wherein the amplified target nucleic acid is detected using a ribonucleotide (RNA) probe labeled with two or more fluorescent substances positioned at a distance that results in a quenching state.

54. A DNA polymerase having a strand displacement activity used in the method for detecting a target nucleic acid according to claim 52 or 53.

55. An endonuclease used in the method for detecting a target nucleic acid according to claim 52 or 53.

56. A kit used for the method for detecting a target nucleic acid according to claim 52 or 53, which is in a packaged form and contains instructions that direct the use of a DNA polymerase and an endonuclease in a strand displacement reaction.

57. The kit according to claim 56, which contains a DNA polymerase and/or an endonuclease.

58. The kit according to claim 57, which is in a package form and contains: (a) a DNA polymerase having a strand displacement activity; (b) an endonuclease; and (c) a buffer for a strand displacement reaction.

59. The kit according to any one of claims 56 to 58, which contains the DNA polymerase having the strand displacement activity according to claim 54 and/or the endonuclease according to claim 55.

60. The kit according to any one of claims 57 to 59, which contains a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax as

61. The kit according to any one claims 57 to 59, which contains RNase H as the endonuclease.

62. A method for producing a material having an immobilized nucleic acid in which the nucleic acid is arrayed in a predefined region, characterized in that the method comprises: (a) amplifying a nucleic acid to be immobilized by the method for amplifying a nucleotide sequence according to any one of claims 1 to 39; and (b) arraying and immobilizing the nucleic acid amplified in step (a) in a predefined region on a substrate.

63. The method according to claim 62, wherein a single-stranded nucleic acid substantially free of a complementary strand thereto is amplified, and arrayed and immobilized in the predefined region on the substrate.

64. A material having an immobilized nucleic acid in which the nucleic acid is arrayed in a predefined region produced by the method according to claim 62 or 63.

65. The material according to claim 64, wherein a single-stranded nucleic acid is arrayed and immobilized in the predefined region.

66. The material according to claim 65, in which a single-stranded nucleic acid substantially free of a complementary strand thereto is arrayed and immobilized in the predefined region.

67. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises: (a) preparing a nucleic acid sample suspected to contain a target nucleic acid from a sample; (b) contacting the nucleic acid sample with the material according to any one of claims 64 to 66; and (c) detecting the target nucleic acid in the nucleic acid sample that hybridizes with the nucleic acid on the material.

68. A method for producing a nucleic acid in large quantities, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide; and (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

69. A method for producing a nucleic acid in large quantities using at least two primers, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide; (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b); (d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with the endonuclease at a site that contains the ribonucleotide; and (f) extending a nucleotide sequence that is

displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

70. A method for producing a nucleic acid in large quantities, characterized in that the method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with the endonuclease; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

71. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) amplifying a nucleic acid containing a sequence to be amplified by a nucleic acid amplification reaction to prepare a nucleic acid as a template; (b) treating the nucleic acid as the template obtained in step (a) with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (c) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (b) with the endonuclease at a site that contains the ribonucleotide; and (d) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (c) to effect a strand displacement.

72. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises: (a) amplifying a nucleic acid containing a sequence to be amplified by a nucleic acid amplification reaction to prepare a nucleic acid as a template; (b) treating the nucleic acid as the template obtained in step (a) with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (c) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (b) with the endonuclease at a site that contains the ribonucleotide; (d) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (c) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (c); (e) treating a released displaced strand obtained in step (d) as a template with at least one primer that is different from that used in step (b) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (b) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease; (f) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (e) with the endonuclease at a site that contains the ribonucleotide; and (g) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (f) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (f).

73. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) amplifying a nucleic acid containing a sequence to be amplified by a nucleic acid amplification reaction to prepare a nucleic acid as a template; (b) preparing a reaction mixture

deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with the endonuclease; and (c) incubating the reaction mixture for a sufficient time to generate a reaction product.

74. The method according to any one of claims 71 to 73, wherein the nucleic acid amplification reaction for preparing the nucleic acid as the template is selected from the group consisting of the TAS method, the 3SR method, the NASBA method, the TMA method, the Qβ replicase method, the PCR method, the LCR method and the SDA method.

75. The method according to claim 74, wherein a random primer or a degenerate primer is used for the nucleic acid amplification reaction.

76. The method according to claim 75, wherein the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

77. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide; and (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

78. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide; (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b); (d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with an endonuclease at a site that contains the ribonucleotide; and (f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

79. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic

ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

80. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein an endonuclease cleaves at a site that contains the ribonucleotide; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at the site that contains the ribonucleotide; and (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

81. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein an endonuclease cleaves at a site that contains the ribonucleotide; (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at the site that contains the ribonucleotide; (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b); (d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein an endonuclease cleaves at a site that contains the ribonucleotide; (e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with the endonuclease at the site that contains the ribonucleotide; and (f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

82. A method for amplifying a nucleotide sequence, characterized in that the method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein the endonuclease cleaves at a site that contains the ribonucleotide; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

83. A method for determining a nucleotide sequence of a nucleic acid, characterized in that the method comprises amplifying a nucleotide sequence according to the method according to any one of claims 1 to 39 and 71 to 82.

84. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase

H, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

85. The method according to claim 84, wherein the reaction mixture further contains a chimeric oligonucleotide primer having a sequence substantially homologous to the nucleotide sequence of the nucleic acid as the template.

86. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template and synthesize a double-stranded nucleic acid, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; and (c) reusing in step (b) the double-stranded nucleic acid obtained in step (b) as a template.

87. A method for amplifying a nucleic acid using at least two primers, characterized in that the method comprises: (a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; (c) reusing in step (b) the double-stranded nucleic acid obtained in step (b) as a template; (d) treating a displaced strand obtained in step (b) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (e) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; and (f) reusing in step (e) the double-stranded nucleic acid obtained in step (e) as a template.

88. The method according to claim 86 or 87, wherein the DNA polymerase is at least one DNA polymerase having a strand displacement activity.

89. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; and

a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the template and the primer-extended strand.

90. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; and (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other.

91. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; (d) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid to which the two primers are annealed obtained in step (c) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; and (e) reusing in step (d) the double-stranded nucleic acid to which the two primers are annealed obtained in step (d).

92. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to

consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; (d) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid to which the two primers are annealed obtained in step (c) to effect a strand displacement and obtain a double-stranded nucleic acid consisting of the template and the primer-extended strand; (e) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the template and the primer-extended strand obtained in step (d) with the endonuclease; and (f) extending a nucleic acid that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to synthesize a displaced strand.

93. The method according to any one of claims 89 to 92, wherein the endonuclease is an endoribonuclease.

94. The method according to claim 93, wherein the endoribonuclease is an RNase H.

95. The method according to any one of claims 84 to 88 and 94, wherein the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Thermotoga, an RNase H from a bacterium of genus Thermus, an RNase H from a bacterium of genus Pyrococcus, an RNase H from a bacterium of genus Archaeoglobus and an RNase H from a bacterium of genus Bacillus.

96. The method according to any one of claims 84 to 95, wherein the length of the region of the nucleic acid to be amplified is 200 bp or shorter.

97. The method according to any one of claims 84 to 96, wherein a chimeric oligonucleotide primer represented by general formula below is used: 5'-dNa-Nb-dNc-3'tm General formula: (a: an integer of 11 or more; b: an integer of 1 or more; c: 0 or an integer of 1 or more; dN: deoxyribonucleotide and/or nucleotide analog; N: unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-terminus by the action of the DNA polymerase does not take place).

98. The method according to claim 97, wherein c is 0.

99. The method according to claim 97 or 98, wherein the nucleotide analog is deoxyriboinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is (α-S) ribonucleotide.

100. The method according to any one of claims 97 to 99, wherein the DNA extension reaction is conducted at a DNA extension reaction temperature suitable for the chimeric oligonucleotide primer as defined in any one of claims 97 to 99.

101. The method according to any one of claims 84 to 100, which comprises annealing the nucleic acid as the template to the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid in an annealing solution containing a substance that enhances the annealing of the nucleic acid to the primer.

102. The method according to claim 101, wherein the annealing solution contains spermidine and/or propylenediamine.

103. The method according to claim 101 or 102, wherein the annealing is conducted by incubating the annealing solution containing the nucleic acid as the template and the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid at 90° C. or above and then cooling the solution to a temperature at which the amplification reaction is conducted or below.

104. The method according to any one of claims 84 to 103, wherein the amplification reaction is conducted in a buffer containing a buffering component selected from the group consisting of Bicine and HEPES.

105. The method according to any one of claims 84 to 104, wherein the DNA polymerase having a strand displacement activity is selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking

106. The method according to any one of claims 84 to 88 and 94 to 105, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and an RNase H from a bacterium of genus Archaeoglobus.

107. The method according to claim 106, wherein the RNase H is type I RNase H from Escherichia coli, or type II RNase H from a bacterium of genus Pyrococcus or a bacterium of genus Archaeoglobus.

108. The method according to any one of claims 84 to 107, wherein a DNA polymerase having an endonuclease activity is used.

109. The method according to claims 108, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the Bca DNA polymerase is used in the presence of a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

110. The method according to claim 109, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

111. The method according to any one of claims 84 to 110, wherein the amplification reaction is conducted in the presence of a substance that inhibits the reverse transcription activity of the DNA polymerase.

112. The method according to claim 111, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

113. The method according any one of claims 84 to 112, wherein the nucleic acid as the template is a single-stranded DNA or a double-stranded DNA.

114. The method according to claim 113, which is conducted after converting a double-stranded DNA as the template into single-stranded DNAs.

115. The method according to claim 113 or 114, wherein the nucleic acid as the template is a cDNA obtained by a reverse transcription reaction using an RNA as a template.

116. The method according to claim 115, which is conducted after synthesizing a cDNA by a reverse transcription reaction using an RNA as a template.

117. The method according to claim 115 or 116, wherein a DNA polymerase having a reverse transcriptase activity is used as a reverse transcriptase.

118. The method according to any one of claims 114 to 117, wherein the reverse transcription reaction and the synthesis of the extended strand that is complementary to the template are conducted using one DNA polymerase having both a reverse transcriptase activity and a strand displacement activity.

119. The method according to claim 118, wherein the DNA polymerase is Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus or Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

120. The method according to any one of claims 84 to 119, wherein the nucleic acid amplification reaction is conducted under isothermal conditions.

121. The method according to any one of claims 84 to 120, wherein the nucleic acid amplification reaction is conducted in the presence of a deoxyribonucleotide triphosphate analog.

122. The method according to claim 121, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

123. A composition for amplifying a nucleic acid which contains: (a) at least one primer that is substantially complementary to a nucleotide sequence of a nucleic acid as a template, wherein the primer is a chimeric oligonucleotide primer that contains a ribonucleotide as well as at least one selected from the group consisitng of a

positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) an endonuclease; and (c) a DNA polymerase having a strand displacement activity.

124. A composition for amplifying a nucleic acid which contains: (a) at least two primers that are substantially complementary to nucleotide sequences of respective strands of a double-stranded nucleic acid as a template, wherein each primer is a chimeric oligonucleotide primer that contains a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; (b) an endonuclease; and (c) a DNA polymerase having a strand displacement activity.

125. A composition for amplifying a nucleic acid obtained by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer.

126. A composition for amplifying a nucleic acid obtained by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least two primers and an endonuclease, wherein each primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of each strand of the double-stranded nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisitng of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer.

127. The composition according to any one of claims 123 to 126, wherein the primer is a chimeric oligonucleotide primer represented by general formula below: 5'-dNa-Nb-dNc-3' General formula: (a: an integer of 11 or more; b: an integer of 1 or more; c: 0 or an integer of 1 or more; dN: deoxyribonucleotide and/or nucleotide analog; N: unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-terminus by the action of the DNA polymerase does not take place).

128. The composition according to claim 127, wherein c is 0.

129. The composition according to claim 127 or 128, wherein the nucleotide analog is deoxyriboinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is (α-S) ribonucleotide.

130. The composition according to any one of claims 123 to 129, which contains a buffering component suitable for a nucleic acid amplification reaction.

131. The composition according to claim 130, which contains a buffering component selected from the group consisting of Bicine and HEPES.

132. The composition according to any one of claims 123 to 131, wherein a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax is used as the DNA polymerase having a strand displacement activity.

133. The composition according to any one of claims 123 to 131, wherein the endonuclease is an endoribonuclease.

134. The composition according to claim 133, wherein the endoribonuclease is an RNase H.

135. The composition according to claim 134, wherein the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Thermotoga, an RNase H from a bacterium of genus Thermus, an RNase H from a bacterium of genus Pyrococcus, an RNase H from a bacterium of genus Archaeoglobus and an RNase H from a bacterium of genus Bacillus.

136. The composition according to any one of claims 123 to 135, wherein the DNA polymerase having a strand displacement activity is Bca DNA

the endonuclease is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and an RNase H from a bacterium of genus Archaeoglobus.

137. The composition according to claim 136, wherein the RNase H is type I RNase H from Escherichia coli, or type II RNase H from a bacterium of genus Pyrococcus or a bacterium of genus Archaeoglobus.

138. The composition according to any one of claims 123 to 137, wherein a DNA polymerase having an endonuclease activity is used.

139. The composition according to claims 138, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax, the composition containing a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

140. The composition according to claim 139, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

141. The composition according to any one of claims 123 to 140, which contains a substance that inhibits the reverse transcription activity of the DNA polymerase.

142. The composition according to claim 141, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

143. The composition according to any one of claims 123 to 142, which contains a deoxyribonucleotide triphosphate analog.

144. The composition according to claim 143, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

145. A composition for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 84 to 88, which contains: (a) an RNase H; and (b) a DNA polymerase having a strand displacement activity.

146. A composition for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 89 to 92, which contains: (a) an endonuclease; and (b) a DNA polymerase having a strand displacement activity.

147. The composition according to claim 146, wherein the endonuclease is an endoribonuclease.

148. The composition according to claim 147, wherein the endoribonuclease is an RNase H.

149. The composition according to claim 145 or 148, wherein the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Thermotoga, an RNase H from a bacterium of genus Thermus, an RNase H from a bacterium of genus Pyrococcus, an RNase H from a bacterium of genus Archaeoglobus and an RNase H from a bacterium of genus Bacillus.

150. The composition according to any one of claims 145 to 149, which contains a buffering component suitable for a nucleic acid amplification reaction.

151. The composition according to claim 150, which contains a buffering component selected from the group consisting of Bicine and HEPES.

152. The composition according to any one of claims 145 to 151, wherein a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax is used as the DNA polymerase having a strand displacement activity.

153. The composition according to claim 145, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and a bacterium of genus Archaeoglobus.

154. The composition according to claim 146, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking

is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and an RNase H from a bacterium of genus Archaeoglobus.

155. The composition according to any one of claims 145 to 154, wherein a DNA polymerase having an endonuclease activity is used.

156. The composition according to claims 155, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax, the composition containing a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

157. The composition according to claim 156, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

158. The composition according to any one of claims 145 to 157, which contains a substance that inhibits the reverse transcription activity of the DNA polymerase.

159. The composition according to claim 158, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

160. The composition according to any one of claims 145 to 159, which contains a deoxyribonucleotide triphosphate analog.

161. The composition according to claim 160, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

162. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 84 to 88, which contains: (a) an RNase H; and (b) a DNA polymerase having a strand displacement activity.

163. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 89 to 92, which contains: (a) an endonuclease; and (b) a DNA polymerase having a strand displacement activity.

164. The kit according to claim 163, wherein the endonuclease is an endoribonuclease.

165. The kit according to claim 164, wherein the endoribonuclease is an RNase H.

166. The kit according to claim 162 or 165, wherein the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Thermotoga, an RNase H from a bacterium of genus Thermus, an RNase H from a bacterium of genus Pyrococcus, an RNase H from a bacterium of genus Archaeoglobus and an RNase H from a bacterium of genus Bacillus.

167. The kit according to any one of claims 162 to 166, which contains a buffer suitable for a nucleic acid amplification reaction.

168. The kit according to claim 167, which contains a buffer for nucleic acid amplification containing a buffering component selected from the group consisting of Bicine and HEPES.

169. The kit according to any one of claims 162 to 168, which contains an annealing solution containing a substance that enhances the annealing of the nucleic acid as the template to the primer that is substantially complementary to the nucleotide sequence of the nucleic acid.

170. The kit according to claim 169, wherein the annealing solution contains spermidine and/or propylenediamine.

171. The kit according to any one of claims 162 to 170, wherein a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax is used as the DNA polymerase having a strand displacement activity.

172. The kit according to claim 162, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and an RNase H from a bacterium of

173. The kit according to claim 163, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the endonuclease is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and an RNase H from a bacterium of genus Archaeoglobus.

174. The kit according to claim 172 or 173, wherein the RNase H is type I RNase H from Escherichia coli, or type II RNase H from a bacterium of genus Pyrococcus or a bacterium of genus Archaeoglobus.

175. The kit according to any one of claims 162 to 174, wherein a DNA polymerase having an endonuclease activity is used.

176. The kit according to claims 175, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax, the kit containing a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

177. The kit according to claim 176, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

178. The kit according to any one of claims 172 to 177, which contains a substance that inhibits the reverse transcription activity of the DNA polymerase.

179. The kit according to claim 178, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

180. The kit according to any one of claims 162 to 179, which contains a deoxyribonucleotide triphosphate analog.

181. The kit according to claim 180, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

182. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 84 to 88, which is in a packaged form and contains instructions that direct the use of a DNA polymerase having a strand displacement activity and an RNase H.

183. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 89 to 92, which is in a packaged form and contains instructions that direct the use of a DNA polymerase having a strand displacement activity and an endonuclease.

184. A product of a reagent for amplifying a nucleic acid consisting of a packing material and a reagent for amplifying a nucleic acid enclosed in the packing material, wherein the reagent for amplifying a nucleic acid contains a DNA polymerase having a strand displacement activity and/or an RNase H, and description that the reagent for amplifying a nucleic acid can be used for nucleic acid amplification under isothermal conditions is indicated in a label stuck to the packaging material or instructions attached to the packaging material.

185. A product of a reagent for amplifying a nucleic acid consisting of a packing material and a reagent for amplifying a nucleic acid enclosed in the packing material, wherein the reagent for amplifying a nucleic acid contains a DNA polymerase having a strand displacement activity and/or an endonuclease, and description that the reagent for amplifying a nucleic acid can be used for nucleic acid amplification under isothermal conditions is indicated in a label stuck to the packaging material or instructions attached to the packaging material.

186. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises: (a) amplifying a nucleic acid by the method for amplifying a nucleic acid according to any one of claims 84 to 122; and (b) detecting a target nucleic acid amplified in step (a).

187. The method according to claim 186, which comprises detecting the amplified nucleic acid using a probe for detection.

188. The method according to claim 187, wherein the probe for detection is a probe that has been labeled with a labeling substance.

189. The method according to claim 188, wherein the probe is an RNA probe labeled with two or more fluorescent substances positioned at a

190. A chimeric oligonucleotide primer used for the method for detecting a target nucleic acid according to any one of claims 186 to 189.

191. The chimeric oligonucleotide primer according to claim 190 which is represented by general formula below: 5'-dNa-Nb-dNc-3' General formula: (a: an integer of 11 or more; b: an integer of 1 or more; c: 0 or an integer of 1 or more; dN: deoxyribonucleotide and/or nucleotide analog; N: unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'terminus by the action of the DNA polymerase does not take place).

192. The chimeric oligonucleotide primer according to claim 191, wherein c is 0.

193. The chimeric oligonucleotide primer according to claim 191 or 192, wherein the nucleotide analog is deoxyriboinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is (α-S) ribonucleotide.

194. The chimeric oligonucleotide primer according to any one of claims 190 to 193, which is a chimeric oligonucleotide primer for detecting a pathogenic microorganism or a disease-related gene.

195. The chimeric oligonucleotide primer according to claim 194, wherein the pathogenic microorganism is enterohemorrhagic Escherichia coli, Clostridium botulinum, Staphylococcus aureus, Mycobacterium tuberculosis, Chlamydia, papilloma virus, hepatitis C virus or a viroid.

196. A chimeric oligonucleotide primer for detecting enterohemorrhagic Escherichia coli having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 31 to 34, 47, 48, 51-53, 64-72, 84, 85, 113, 114, 130 and 131.

197. A chimeric oligonucleotide primer for detecting a viroid having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 59, 60, 119, 120, 122 and 123.

198. A chimeric oligonucleotide primer for detecting Clostridium botulinum having a nucleotide sequence represented by SEQ ID NO: 116 or 117.

199. A chimeric oligonucleotide primer for detecting papilloma virus having a nucleotide sequence represented by SEQ ID NO: 96 or 97.

200. A chimeric oligonucleotide primer for detecting hepatitis C virus having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 101, 102, 138, 139, 200, 201, 205 and 206.

201. A chimeric oligonucleotide primer for detecting Staphylococcus aureus having a nucleotide sequence represented by SEQ ID NO: 136 or 137.

202. A chimeric oligonucleotide primer for detecting Mycobacterium tuberculosis having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 155, 156, 159 to 162, 194 and 195.

203. A chimeric oligonucleotide primer for detecting Chlamydia having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 157, 158, 203 and 204.

204. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 84 to 122, which contains the chimeric oligonucleotide primer according to any one of claims 190 to 203.

205. A kit for detecting a target nucleic acid used for the method for detecting a target nucleic acid according to any one of claims 186 to 189, which contains the chimeric oligonucleotide primer according to any one of claims 190 to 203.

206. A probe used in the method according to any one of claims 186 to 189.

207. A probe which hybridizes to the nucleic acid amplified by the method according to any one of claims 84 to 122.

208. A probe which hybridizes to a region amplified using the chimeric oligonucleotide primer according to any one of claims 196 to 203.

209. The probe according to any one of claims 206 to 208, which has been

210. The probe according to claim 209, which is an RNA probe labeled with two or more fluorescent substances positioned at a distance that results in a quenching state

211. A kit used in the method according to any one of claims 186 to 189, which contains the probe according to any one of claims 206 to 210.

212. A method for amplifying a nucleic acid, which comprises using a DNA polymerase having a strand displacement activity to effect a template switching reaction.

213. The method according to claim 212, wherein the DNA polymerase having a strand displacement activity is selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

214. A method for producing a material having an immobilized nucleic acid in which the nucleic acid is arrayed in a predefined region, characterized in that the method comprises: (a) amplifying a nucleic acid to be immobilized by the method for amplifying a nucleic acid according to any one of claims 84 to 122; and (b) arraying and immobilizing the nucleic acid amplified in step (a) in a predefined region.

215. A material having an immobilized nucleic acid in which the nucleic acid is arrayed in a predefined region produced by the method according to claim 214.

216. A method for producing a nucleic acid in large quantities, characterized in that the method comprises: (a) amplifying a nucleic acid by the method for amplifying a nucleic acid according to any one of claims 84 to 122; and (b) collecting the nucleic acid amplified in step (a).

217. A method for amplifying a nucleic acid, characterized in that the method comprises: (a) duplicating a DNA or an RNA containing a sequence to be amplified to prepare a nucleic acid as a template; and (b) amplifying the nucleic acid as the template obtained in step (a) by the method for amplifying a nucleic acid according to any one of claims 84 to 122.

218. A method for determining a nucleotide sequence of a nucleic acid, characterized in that the method comprises amplifying a nucleic acid according to the method according to any one of claims 84 to 122, 216 and 217.

219. A method for preparing a single-stranded nucleic acid, the method comprising a step of generating a single-stranded nucleic acid using the method according to any one of claims 84 to 122.

220. The method according to claim 219, wherein at least two primers at different concentrations are used.

L17 ANSWER 9 OF 12 USPATFULL on STN
2002:283158 Methods for **retroviral** mediated gene transfer employing molecules, or mixtures thereof, containing **retroviral** binding domains and target cell binding domains.
Asada, Kiyozo, 3-20-9, Kibogaoka, Konan-cho, Koka-gun, Shiga/520-33, JAPAN
Uemori, Takashi, Sharumankorpo Daini Seta 709, 1-16, Oe 3-chome, Otsu-shi, Shiga/520-21, JAPAN
Ueno, Takashi, Hamoparesu Kusatsu 609, 12-1, Nishishibukawa 2-chome, Kusatsu-shi, Shiga 525, JAPAN
Koyama, Nobuto, 96, Kubo, Ogura-cho, Uji-shi, Kyoto 611, JAPAN
Hasino, Kimakazu, 27-3, Akeno-cho, Takatsuki-shi, Osaka 569, JAPAN
Kato, Ikunoshin, 1-1-150, Nanryo-cho, Uji-shi, Kyoto 611, JAPAN
US 6472204 B1 20021029
WO 9718318 19970522
APPLICATION: US 1997-809156 19970307 (8)
WO 1996-JP3254 19961107
PRIORITY: JP 1995-294382 19951113
JP 1996-51847 19960308
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method is disclosed for increasing the efficiency of **retroviral** mediated gene transfer into viable target cells, which comprises transducing the target cells by infecting the target cells with a replication defective recombinant **retrovirus** that infects the target cells in an aqueous medium in the presence of

(a) a mixture of an effective amount of a first functional material having a **retrovirus** binding domain that binds said **retrovirus**, and an effective amount of a second functional material having a target cell binding domain that binds said target cell, or

(b) an effective amount of a bifunctional material having both a **retroviral** binding domain which does not contain the heparin binding domain derived from human fibronectin, and a target cell binding domain, wherein the bifunctional material has a **retrovirus** binding domain that binds to said **retrovirus** and a target cell binding domain that binds to the target cell.

CLM What is claimed is:

1. An ex vivo method for increasing the efficiency of **retroviral** mediated gene transfer into viable target cells, which comprises transducing the target cells by infecting said target cells with a replication defective recombinant **retrovirus** that infects said target cells in an aqueous medium in the presence of (a) a mixture of an effective amount of a first functional material having a **retrovirus** binding domain that binds said **retrovirus** and an effective amount of a second functional material having a target cell binding domain that binds said target cell, or (b) an effective amount of a bifunctional material having both a **retroviral** binding domain which does not contain the heparin binding domain derived from human fibronectin, and a target cell binding domain, wherein said bifunctional material has a **retrovirus** binding domain that binds said **retrovirus** and a target cell binding domain that binds said target cell.

2. The method of claim 1 wherein the transduction of the target cells is performed with a mixture of an effective amount of a polypeptide having a **retrovirus** binding domain, and an effective amount of a functional material having a target cell binding domain, said target cell binding domain being selected from the group consisting of cell adhesion proteins, hormones, cytokines, antibodies, carbohydrates, a cell binding domain of fibronectin, and derivatives thereof.

3. The method of claim 1 wherein the transduction of the target cells is performed with a polypeptide which has both a **retrovirus** binding domain which is not derived from the heparin binding domain of human fibronectin, and a target cell binding domain, said target cell binding domain being selected from the group consisting of cell adhesion proteins, hormones, cytokines, antibodies, carbohydrates, a cell binding domain of fibronectin, and derivatives thereof.

4. The method of claim 3 wherein the functional material is not immobilized.

5. The method of claim 1 wherein said target cells are selected from the group consisting of adherent cells, bone marrow cells, hematopoietic stem cells, myeloid stem cells, peripheral blood stem cells, umbilical blood cells, embryonic cells, CD34+ cells, unipotential hematopoietic progenitor cells, erythrocyte precursor cells, lymphocytes, T cells, fibroblasts, hepatic cells, and cancer cells.

6. The method of claim 2 wherein the transduction of the target cells is performed with a mixture of materials having the functional domains, the **retrovirus** binding domain being selected from the group consisting of the Heparin-II binding domain of fibronectin, fibroblast growth factor, collagen and fragments thereof, and a polylysine, and the target cell binding domain being selected from the group consisting of cell adhesion proteins, hormones, cytokines, antibodies, carbohydrates and derivatives thereof.

7. The method of claim 3 wherein the transduction of the target cells is performed with a polypeptide which has both a **retrovirus** binding domain and a target cell binding domain, the retorvirus binding domain being selected from the group consisting of fibroblast growth factor, collagen and fragments thereof and polylysine, and the target cell binding domain being selected from the group consisting of cell adhesion proteins, hormones, cytokines, antibodies, carbohydrates, a cell binding domain of fibronectin, and derivatives thereof.

8. The method of claim 6 wherein the **retrovirus** binding domain is selected from the group of polypeptides consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:13, and SEQ ID NO:28 and the polypeptide comprising the target cell binding domain is selected from the group of polypeptides consisting of SEQ ID NO:2, SEQ: 25, SEQ ID NO:29, and SEQ ID NO: 34.

9. The method of claim 6 wherein the **retrovirus** binding domain comprises SEQ ID NO:1.

10. The method of claim 6 wherein the **retrovirus** binding domain is a

11. The method of claim 6 wherein the **retrovirus** binding domain is a portion of fibroblast growth factor comprising SEQ ID NO:3.

12. The method of claim 6 wherein the **retrovirus** binding domain is a portion of collagen V comprising SEQ ID NO:6.

13. The method of claim 6 wherein the **retrovirus** binding domain is a ColV fragment comprising SEQ ID NO:28.

14. The method of claim 6 wherein the **retrovirus** binding domain is a polymer of L-lysine.

15. The method of claim 7 wherein the functional material is a polypeptide comprising SEQ ID NO:4.

16. The method of claim 7 wherein the functional material is a polypeptide comprising SEQ ID NO:5.

17. The method of claim 7 wherein the functional material is a polypeptide comprising SEQ ID NO:7.

18. The method of claim 7 wherein the functional material is a polypeptide comprising SEQ ID NO:8.

19. The method of claim 4 wherein said bifunctional material is a polypeptide comprising SEQ ID NO:14.

20. The method of claim 4 wherein said bifunctional material is a polypeptide comprising SEQ ID NO:30.

21. The method of claim 4 wherein said bifunctional material is a polypeptide comprising SEQ ID NO:21.

22. The method of claim 4 wherein said bifunctional material is a polypeptide comprising SEQ ID NO:22.

23. The method of claim 4 wherein said bifunctional material is a polypeptide comprising SEQ ID NO:23.

24. The method of claim 4 wherein said bifunctional material is a polypeptide comprising SEQ ID NO:24.

25. The method of claim 6 wherein the **retrovirus** binding domain is fibroblast growth factor and the functional material which has a target cell binding domain is a polypeptide comprising SEQ ID NO:25.

26. The method of claim 6 wherein the materials having functional domains are not immobilized.

27. The method of claim 6 wherein the materials having functional domains are immobilized.

28. The method of claim 1 wherein said replication defective recombinant **retrovirus** comprises an exogenous gene.

29. The method of claim 6 wherein the mixture of materials having functional domains is immobilized on beads.

30. The method of claim 7 wherein the material having functional domains is immobilized on beads.

31. The method of claim 6 wherein the target cell binding domain is selected to be specific for the target cell.

32. The method of claim 1 wherein said first functional material having a **retrovirus** binding domain is fibroblast growth factor and said second functional material having a target cell binding domain is at least one cytokine.

33. The method of claim 6 wherein the target cells are selected from the group consisting of hematopoietic pluripotent stem cells, lymphoid stem cells, myeloid stem cells, embryoplastic stem cells, and embryonic stem cells.

34. The method of claim 7 wherein the target cells are selected from the group consisting of hematopoietic pluripotent stem cells, lymphoid stem cells, myeloid stem cells, embryoplastic stem cells, and embryonic stem cells.

utilizing molecules, or mixture thereof, containing **retroviral** binding domains and target cell binding domains.

Asada, Kiyozo, Shiga, JAPAN
Uemori, Takashi, Otsu, JAPAN
Ueno, Takashi, Kusatsu, JAPAN
Koyama, Nobuto, Uji, JAPAN
Hashino, Kimikazu, Takatsuki, JAPAN
Kato, Ikunoshin, Uji, JAPAN
Takara Shuzo Co., Ltd., JAPAN (non-U.S. corporation)

US 6426042 B1 20020730
APPLICATION: US 1999-366009 19990802 (9)
PRIORITY: JP 1995-294382 19951113
JP 1996-51847 19960308
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a kit to carry out **retrovirus**-mediated gene transfer into target cells. The kit contains a functional material bearing a **retrovirus** binding domain, another functional material bearing a target cell binding domain, an artificial substrate for incubating the **retrovirus** contacted with the target cells, and a target cell growth factor for pre-stimulating target cells to spur them along the cell cycle. The kit of the invention may further comprise a recombinant **retroviral** vector, necessary buffers, and the like.

CLM What is claimed is:

1. A kit for carrying out **retrovirus** mediated gene transfer into target cells comprising a mixture of an effective amount, of (a) an isolated polypeptide having a **retroviral** binding domain that binds said **retrovirus** and (b) an isolated ligand having a target cell binding domain that binds said target cell, an artificial substrate for incubating the **retrovirus** with the target cell, and a growth factor competent for inducing entry of the target cell into the cell cycle.

2. The kit of claim 1 wherein the **retroviral** binding domain of said isolated polypeptide is selected from the group consisting of the Heparin-II binding domain of fibronectin, fibroblast growth factors (FGF) collagen and fragments thereof, and polylysine.

3. The kit of claim 2 wherein said isolated polypeptide comprising the **retroviral** binding domain is selected from the group of polypeptides consisting of SEQ ID Nos. 1, 3, 6, 13 and 28.

4. The kit of claim 2 wherein the **retroviral** binding domain is the Heparin-II binding domain of human fibronectin, referred to as H-271 (SEQ ID No. 1).

5. The kit of claim 2 wherein the **retroviral** binding domain is the polypeptide comprising two Heparin-II binding domains of human fibronectin, referred to as H2-547 (SEQ ID No. 13).

6. The kit of claim 2 wherein the **retroviral** binding domain is a portion of FGF (SEQ ID No. 3).

7. The kit of claim 2 wherein the **retroviral** binding domain is a portion of Collagen V which has insulin binding activity, referred to as ColV (SEQ ID No. 6).

8. The kit of claim 2 wherein the **retroviral** binding domain is a portion of ColV which has insulin-binding activity (SEQ ID No. 28).

9. The kit of claim 2 wherein the **retroviral** binding domain is polylysine.

10. The kit of claim 1 wherein said isolated polypeptide and said isolated ligand are immobilized.

11. The kit of claim 10 wherein said isolated polypeptide and said ligand are immobilized onto beads.

12. The kit of claim 10 wherein said isolated polypeptide and said ligand are not immobilized.

13. The kit of claim 10 wherein the target cell binding domain of the said ligand is selected from the group consisting of cell adhesion proteins, hormones, cytokines, antibodies, glycoproteins, glycolipids, carbohydrates and metabolic intermediates of the target cell.

14. The kit of claim 13 wherein the target cell binding domain of said ligand is selected from the group of polypeptides consisting of SEQ ID Nos. 2, 25, 29, and 34.

15. A kit for carrying out **retrovirus** mediated gene transfer into target cells comprising an isolated molecule having both a **retroviral**

binding domain, an artificial substrate for incubating the **retrovirus** with the target cell, and a growth factor competent for inducing entry of the target cell into the cell cycle.

16. The kit of claim 11 wherein the **retroviral** binding domain is selected from the group of polypeptides consisting of FGF, collagen and fragments thereof and polylysine.

17. The kit of claim 15 wherein the **retroviral** binding domain is selected from the group of compounds consisting of cell adhesion proteins, hormones, cytokines, glycoproteins, glycolipids, fibronectin, and metabolic intermediates of the target cell.

18. The kit of claim 15 wherein said molecule is immobilized.

19. The kit of claim 15 wherein said molecule is immobilized onto beads.

20. The kit of claim 15 wherein said molecule is not immobilized.

21. The kit of claim 15 wherein said molecule is a polypeptide referred to as C-FGF.A or as C-274-FGF (SEQ ID No. 4), which comprises a **retroviral** binding domain consisting of the N-terminal portion of FGF (SEQ ID No. 3) fused to the target cell binding domain of fibronectin corresponding to the sequence $PRO^{1239-Ser1515}$ thereof, (SEQ ID No. 25).

22. The kit of claim 15 wherein said molecule is C-FGF-CSI (SEQ ID No. 5), which comprises C-FGF.A fused to the CS1 target cell binding domain of human fibronectin (SEQ ID No. 2).

23. The kit of claim 15 wherein said molecule is a polypeptide C277-ColV (SEQ ID No. 7), which comprises a **retroviral** binding domainconsisting of the portion of Collagen V (SEQ ID No. 6) and the target cell binding domain of fibronectin, corresponding to Sequence $Pro^{1239-Ser1515}$ thereof.

24. The kit of claim 15 wherein said molecule is C-ColV-Cs1 (SEQ ID No. 8), which comprises C277-ColV fused to the CS1 target cell binding domain of human fibronectin (SEQ ID No. 2), wherein the second glutamic acid from the C-terminus of ColV and the C-terminal threonine are replaced by alanine and serine, respectively.

25. A kit for carrying out **retroviral** mediated gene transfer into target cells comprising non-immobilized fragments of fibronectin, said fragments comprising the Heparin-II virus binding region and the target cell binding region of human fibronectin, an artificial substrate for incubating the **retrovirus** with the target cell and a growth factor competent for inducing entry of the target cell into the cell cycle.

26. The kit of claim 25 wherein the fibronectin fragment is CH2-826 (SEQ ID No. 14), which comprises two Heparin-II virus binding domains of fibronectin coupled to C-274, the target cell binding domain of fibronectin.

27. The kit of claim 25 wherein the fibronectin fragment is H2S-573 (SEQ ID No. 30), which comprises the H2547 virus binding region (SEQ ID No. 13) coupled to the CS1 target cell binding region of fibronectin (SEQ ID No. 2).

28. The kit of claim 25 wherein the fibronectin fragment is CHV-181 (SEQ ID No. 21), which comprises a target cell binding domain of fibronectin ($Pro^{1239-Ser1515}$) to which C-terminal is added via methionine, the Type III repeats 12 and 13 of the Heparin-II virus binding domain.

29. The kit of claim 25 wherein the fibronectin fragment is CHV-179 (SEQ ID No. 22), which comprises a target cell binding domain of fibronectin ($Pro^{1239-Ser1515}$) to which C-terminal is added via methionine, the Type III repeats 13 and 14 of the Heparin-II virus binding domain.

30. The kit of claim 25 wherein the fibronectin fragment is CHV-271 (SEQ ID No. 23), which comprises the fibronectin binding virus domain (SEQ ID No. 1) coupled to C-274 (SEQ ID No. 25) which represents the fibronectin binding domain that binds to VLA5.

31. The kit of claim 25 wherein the fibronectin fragment is CH-296 (SEQ ID No. 24), which comprises the fibronectin virus binding fragment H-296 coupled to C-274.

32. The kit of claim 1 wherein the components are freeze dried, in the form of granules, tablets or in an aqueous medium.

33. The kit of claim 15 wherein the components are freeze dried, in the

34. The kit of claim 25 wherein the components are freeze dried, in form of granules, tablets or aqueous medium.

35. The kit of claim 1 wherein said isolated polypeptide having a **retroviral** binding domain is FGF and said ligand having a target cell binding domain is cytokine.

36. The kit of claim 15 wherein said **retroviral** binding domain is FGF and said target cell binding domain is cytokine.

37. The kit of claim 25 wherein the functional material having a **retrovirus** binding domain is FGF and the functional material having a target cell binding domain is cytokine.

38. The kit of claim 25 wherein the virus binding domain is referred to as H2-547 (SEQ ID No. 13), which comprises two Heparin-II virus binding domains of fibronectin.

39. The kit of claim 1 wherein only said isolated polypeptide having a **retroviral** binding domain selected from the group of fibronectin fragments consisting of H2-547, CH2-826, H2S-573, CHV-181, and CHV-179 (SEQ ID Nos. 13, 14, 30, 21, and 22, respectively) is immobilized.

40. The kit of claim 39 wherein the fragment is the H2-547 fragment.

41. The kit of claim 39 wherein the fragment is the CH2-826 fragment.

42. The kit of claim 39 wherein the fragment is the H2S-573 fragment.

43. The kit of claim 39 wherein the fragment is the CHV-181 fragment.

44. The kit of claim 39 wherein the fragment is the CHV-179 fragment.

L17 ANSWER 11 OF 12 USPATFULL on STN
2001:145074 Cell compositions.
Asada, Kiyozo, Koka-gun, Japan
Konishi, Haruko, Kyoto-shi, Japan
Koyama, Nobuto, Uji-shi, Japan
Kato, Ikunoshin, Uji-shi, Japan
Takara Shuzo Co., Ltd., Kyoto-shi, Japan (non-U.S. corporation)
US 2001018209 A1 20010830
APPLICATION: US 2001-797821 A1 20010305 (9)
PRIORITY: JP 1996-180500 19960710
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Cell compositions from which cancer cells have been selectively eliminated by using apoptosis inducers specific to cancer cells.
CLM What is claimed is:
1. A cell composition comprising hematopoietic stem cells from which cancer cells have been substantially eliminated, said composition being obtained by selectively eliminating cancer cells with an apoptosis inducer specific to cancer cells, said cancer cells being not genetically engineered, wherein said apoptosis inducer comprises a compound selected from the group consisting of a fucoidan, a decomposition product of a fucoidan, a dextran sulfate, a decomposition product of a dextran sulfate, a heat treated product of a saccharide compound containing a uronic acid and/or a uronic acid derivative, and 4,5-dihydroxy-2-cyclopenten-1-one represented by the formula: ##STR7##

2. A process for obtaining a cell composition comprising hematopoietic stem cells from which cancer cells have been substantially eliminated, said process comprising the steps of: (a) obtaining hematopoietic stem cells from the appropriate source; (b) adding an apoptosis inducer to said hematopoietic stem cells; and (c) inducing apoptosis of cancer cells, said cancer cells being not genetically engineered, wherein said apoptosis inducer comprises a compound selected from the group consisting of a fucoidan, a decomposition product of a fucoidan, a dextran sulfate, a decomposition product of a dextran sulfate, a heat treated product of a saccharide compound containing a uronic acid and/or a uronic acid derivative, and 4,5-dihydroxy-2-cyclopenten-1-one represented by the formula: ##STR8##

3. A cell composition comprising hematopoietic stem cells and as exogenous gene being transferred into said hematopoietic cells, from which cancer cells have been sbstantially eliminated by selectively eliminating cancer cells with an apoptosis inducer specific to cancer cells, said cancer cells being not genetically engineered, wherein said apoptosis inducer comprises a compound selected from the group consisting of a fucoidan, a decomposition product of a fucoidan, a dextran sulfate, a decomposition product of a dextran sulfate, a heat

a uronic acid derivative, and 4, 5-dihydroxy-2-cyclopenten-1-one represented by the formula: ##STR9##

4. A process for obtaining a cell composition comprising hematopoietic stem cells and an exogenous gene being transferred into said hematopoietic stem cells, from which cancer cells have been substantially eliminated, said process comprising the steps of: (a) obtaining hematopoietic stem cells from the appropriate source; (b) adding an apoptosis inducer to said hematopoietic stem cells; and (c) inducing apoptosis of cancer cells, said cancer cells being not genetically engineered, wherein said apoptosis inducer comprises a compound selected from the group consisting of a fucoidan, a decomposition product of a fucoidan, a dextran sulfate, a decomposition product of a dextran sulfate, a heat treated product of a saccharide compound containing a uronic acid and/or a uronic acid derivative, and 4,5-dihydroxy-2-cyclopenten-1-one represented by the formula: ##STR10##

L17 ANSWER 12 OF 12 USPATFULL on STN
2001:29345 Method of purifying and removing viruses.
Ueno, Takashi, Otsu, Japan
Hashino, Kimikazu, Otsu, Japan
Asada, Kiyozo, Otsu, Japan
Kato, Ikunoshin, Otsu, Japan
Takara Shuzo Co., Ltd., Kyoto, Japan (non-U.S. corporation)
US 6194192 B1 20010227
WO 9732010 19970904
APPLICATION: US 1998-117122 19980723 (9)
WO 1997-JP457 19970219 19980723 PCT 371 date 19980723 PCT 102(e) date
PRIORITY: JP 1996-67514 19960229
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention offers a method for the purification or removal of virus characterized in containing a step where the virus in the virus-containing sample is adsorbed with sulfated-fucose-containing polysaccharide(s) and/or degradation product(s) thereof.
CLM What is claimed is:
1. A method for purifying a virus comprising absorbing the virus from a virus-containing sample with sulfated-fucose-containing polysaccharide(s) from Kjellmaniae crassifolia.

2. A method for removing a virus comprising absorbing the virus from a virus-containing sample with sulfated-fucose-containing polysaccharide(s) from Kjellmaniae crassifolia.

3. The method as set forth in claim 1 or 2, wherein said sulfated-fucose-containing polysaccharide(s) is/are (1) substantially free from uronic acid and (2) cannot substantially be degraded by a fucoidanase produced by Flavobacterium sp. SA-0082 (FERM BP-5402).

4. The method as set forth in claim 1 or 2, wherein said virus is a **retrovirus**, adenovirus, adeno-associated virus, baculovirus, influenza virus or herpes virus.

5. The method as set forth in claim 1 or 2, wherein said virus-containing sample is a liquid or a gas.

6. The method as set forth in claim 1 or 2, wherein said sulfated-fucose-containing polysaccharide(s) is/are immobilized on a carrier.

7. The method as set forth in claim 6, wherein said carrier is in the form of a gel or particles.

8. The method as set forth in claim 6, wherein said carrier is in the form of a thin film.

9. The method as set forth in claim 6, wherein said carrier is in the form of a hollow fiber.

10. A composition for absorbing a virus comprising sulfated-fucose-containing polysaccharide(s) from Kjellmaniae crassifolia.

11. The composition as set forth in claim 10, wherein said sulfated-fucose-containing polysaccharide(s) is/are immobilized on a carrier.

12. The composition as set forth in claim 10 or 11, wherein said sulfated-fucose-containing polysaccharide(s) is/are (1) substantially free from uronic acid and (2) cannot substantially be degraded by a fucoidanase produced by Flavobacterium sp. SA-0082 (FERM BP-5402).

13. The composition as set forth in claim 11, wherein said carrier is in the form of a gel or particles.

14. The composition as set forth in claim 11, wherein said carrier is in the form of a thin film.

15. The composition as set forth in claim 11, wherein said carrier is in the form of a hollow fiber.

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=> d his

     (FILE 'HOME' ENTERED AT 23:13:49 ON 19 MAR 2006)

     FILE 'USPATFULL' ENTERED AT 23:14:08 ON 19 MAR 2006
                E HUMEAU LAURENT/IN
L1            6 S E3
                E HAN WEI/IN
L2           47 S E3
L3           45 S L2 NOT L1
L4            2 S L3 AND (RETROVIR? OR LENTIVIR?)
                E LU XIAOBIN/IN
L5            4 S E3
L6            2 S L5 NOT L1
                E SLEPUSHKIN VLADIMIR/IN
L7            5 S E3
L8            2 S L7 NOT L1
                E LESHER MECHELLE/IN
L9            2 S E3
                E DAVIS BRIAN/IN
L10          14 S E3
L11          11 S L10 NOT (L1 OR L2 OR L5 OR L7 OR L9)
L12           0 S L11 AND (RETROVIR? OR LENTIVIR?)
                E DROPULIC BORO/IN
L13          19 S E3
L14          12 S L13 NOT (L1 OR L2 OR L5 OR L7 OR L9)
L15          12 S L14 AND (RETROVIR? OR LENTIVIR?)
                E ASADA KIYOZO/IN
L16          51 S E3
L17          12 S L16 AND (RETROVIR? OR LENTIVIR?)

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 23:33:13 ON 19 MAR 2006
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AMENDMENTS TO THE CLAIMS

Please amend the claims as follows. 1-30, 33-64, 66-71, 83-96

This listing of claims will replace all prior versions, and listing, of claims in the application:

Claim 1 (previously presented): A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells comprising contacting the surface of said primary cell or hematopoietic stem cells at the same time *in vitro* or *ex vivo* with both a lentiviral vector and at least one molecule which binds said cell surface

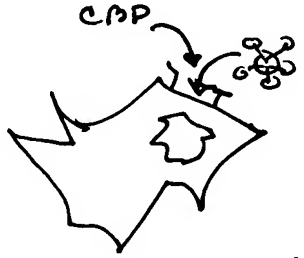
wherein at least about 75% of the cells are stably transduced after about seven to ten days, or at about 14 days.

Claim 2 (previously presented): The method of claim 1 further comprising continuous contacting the primary cells or hematopoietic stem cells *in vitro* or *ex vivo* with the lentiviral vector after the simultaneous contacting of the primary cells or hematopoietic stem cells with the lentiviral vector and the at least one cell surface binding molecule.

Claim 3 (previously presented): The method of claim 1 further comprising continuous contacting the primary cells or hematopoietic stem cells *in vitro* or *ex vivo* with the at least one cell surface binding molecule after the simultaneous contacting of the primary cells or hematopoietic stem cells with the lentiviral vector and the at least one cell surface binding molecule.

Claim 4 (previously presented): The method of claim 1 further comprising continuous contacting the primary cells or hematopoietic stem cells *in vitro* or *ex vivo* with the lentiviral vector and the at least one cell surface binding molecule after the initial simultaneous contact of the primary cells or hematopoietic stem cells with the lentivirus vector and the at least one cell surface binding molecule.

GROUP I : 1-30, 33-64, 66-71



Claim 5 (original): The method of claim 1 where said contacting with a lentiviral vector occurs more than once.

Claim 6 (previously presented): The method of claim 1 wherein said lentiviral vector is derived from a human immunodeficiency virus (HIV).

Claim 7 (previously presented): The method of claim 1 wherein said cell surface binding molecule is an antibody, an antigen binding fragment, a ligand or a cell surface molecule.

Claim 8 (original): The method of claim 1 wherein said lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, vpr, vpu, tat or rev genes.

Claim 9 (previously presented): The method of claim 8 wherein said cis-acting nucleotide sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, vpr, vpu, tat or rev genes.

Claim 10 (previously presented): The method of claim 1 wherein said lentiviral vector is derived from HIV-1 or HIV-2.

Claim 11 (original): The method of claim 1 wherein said lentiviral vector is a pseudotyped vector.

Claim 12 (previously presented): The method of claim 11 wherein said pseudotyped vector comprises the vesicular stomatitis virus G envelope protein.

Claim 13 (previously presented): The method of claim 1 wherein said lentiviral vector is a chimeric vector comprising HIV sequences, wherein optionally the HIV sequences comprise HIV-1 and HIV-2 sequences.

Claim 14 (previously presented): The method of claim 1 wherein said primary hematopoietic cell is a CD4 positive cell or is a hematopoietic stem cell of a CD4 positive cell.

Claim 15 (previously presented): The method of claim 1 wherein said primary cell of the hematopoietic system or hematopoietic stem cell is a lymphocyte or a precursor thereof.

Claim 16 (previously presented): The method of claim 1 wherein the primary cell of the hematopoietic system or hematopoietic stem cell is a CD4 or CD8 positive cell or a precursor thereof.

Claim 17 (previously presented): The method of claim 1 wherein said primary cell of the hematopoietic system or hematopoietic stem cell is a CD34 positive cell or a precursor thereof.

Claim 18 (previously presented): The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from the group consisting of an FLT-3 ligand; a TPO ligand Kit ligand; antibodies that have the same cell surface binding specificity as FLT-3, TPO, or Kit ligand; CD3 ligand; a CD28 ligand; a CD25 ligand; a CD71 ligand; a CD69 ligand; and, antibodies that have are the same cell surface binding specificity of CD3, CD25, CD28, CD69 or CD71 ligand.

Claim 19 (previously presented): The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from the group consisting of FLT-3 ligand, TPO ligand and Kit ligand or polypeptides or other binding molecules that have the same cell surface binding specificity as FLT-3 ligand, TPO ligand, or Kit ligand.

Claim 20 (previously presented): The method of claim 1 wherein the said primary cell or hematopoietic stem cell is a dendritic cell or a cell capable of differentiating into a dendritic cell or a precursor thereof.

Claim 21 (previously presented): The method of claim 1 wherein said at least one cell surface binding molecule is selected from the group consisting of compositions comprising CD34, CD3, CD28, GM-CSF, IL-4, TNF-alpha; GM-CSF, interferon-alpha; and antibodies or other binding molecules that have the same cell surface binding specificity as CD34, CD3, CD28, GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha.

Claim 22 (previously presented): The method of claim 1, wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 antibodies and cell surface binding fragments thereof, CD28 antibodies and cell surface binding fragments thereof, combinations of said antibodies and cell surface binding fragments thereof, and binding molecules that have the same cell surface binding specificities as the antibodies~~, and optionally at least two of the cell surface binding molecules are immobilized on a bead or a surface~~.

Claim 23 (previously presented): The method of claim 22 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 antibodies immobilized on a bead or a surface, wherein optionally the bead or surface comprises coated beads.

Claim 24 (previously presented): The method of claim 1 culturing the primary cells or hematopoietic stem cells under conditions conducive to growth and/or proliferation.

Claim 25 (previously presented): The method of claim 24 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

Claim 26 (original): The method of claim 25 wherein said cytokine is interleukin-2.

Claim 27 (original): The method of claim 24 wherein said culturing is for about seven days.

Claim 28 (original): The method of claim 24 wherein said culturing is for about 14 days.

Claim 29 (previously presented): The method of claim 1 wherein said contacting the primary cells or hematopoietic stem cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

Claim 30 (original): The method of claim 1 wherein the lentiviral vector is present at an MOI of less than 500.

Claims 31 and 32 (canceled)

Claim 33 (original): The method of claim 1 wherein said contacting occurs *ex vivo*.

N281 WD N282

Claim 34 (currently amended): A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells comprising

(a) isolating from an individual a primary cell of the hematopoietic system and/or a hematopoietic stem cell; and

(b) contacting the primary cell or hematopoietic stem cell simultaneously *in vitro* or *ex vivo* with a lentiviral vector and an at least one molecule that physically interacts with a receptor, marker, or other recognizable moiety on the surface of the primary cell or hematopoietic stem cell,

wherein greater than about 75% of the primary cells or hematopoietic stem cells are stably transduced after about seven to ten days, or at about 14 days;

~~and optionally the cell surface binding molecule comprises a polypeptide, a lipid, a nucleic acid, a carbohydrate or an ion~~.

Claim 35 (previously presented): The method of claim 34 further comprising continuous contacting the primary cells or hematopoietic stem cells *in vitro* or *ex vivo* with the lentiviral vector after the simultaneous contacting of the primary cells with the lentiviral vector and the at least one cell surface binding molecule.

Claim 36 (previously presented): The method of claim 34 further comprising continuous contacting the primary cells or hematopoietic stem cells *in vitro* or *ex vivo* with the at least one cell surface binding molecule after the simultaneous contacting of the ~~a~~ lentiviral vector and the at least one cell surface binding molecule.

Claim 37 (previously presented): The method of claim 34 further comprising continuous contacting the primary cells or hematopoietic stem cells *in vitro* or *ex vivo* with the lentiviral vector and the at least one cell surface binding molecule after the initial simultaneous contact of the lentivirus vector and the at least one cell surface binding molecule.

Claim 38 (previously presented): The method of claim 34 wherein ~~where~~ said contacting with a lentiviral vector occurs more than once.

Claim 39 (previously presented): The method of claim 34 wherein said cells are human primary cells of the hematopoietic system and/or human hematopoietic stem cells.

Claim 40 (previously presented): The method of claim 34 wherein said cell surface binding molecule is an antibody, an antigen binding fragment, a ligand or a cell surface molecule.

Claim 41 (previously presented): The method of claim 34 wherein said lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, vpr, vpu, tat or rev genes.

Claim 42 (previously presented): The method of claim 41, wherein said cis-acting nucleotide sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, vpr, vpu, tat or rev genes.

Claim 43 (previously presented): The method of claim 34 wherein said lentiviral vector is an HIV-derived vector.

Claim 44 (previously presented): The method of claim 34 wherein said lentiviral vector is a pseudotyped vector.

Claim 45 (previously presented): The method of claim 44 wherein said pseudotyped vector contains the vesicular stomatitis virus G envelope protein.

Claim 46 (previously presented): The method of claim 34 wherein said primary cell or hematopoietic stem cell is a primary human cell or a human hematopoietic stem cell.

Claim 47 (previously presented): The method of claim 34 wherein said primary cell of the hematopoietic system or hematopoietic stem cell is a CD4 positive cell.

Claim 48 (previously presented): The method of claim 34 wherein said primary cell of the hematopoietic system or hematopoietic stem cell is a lymphocyte or a precursor thereof.

Claim 49 (previously presented): The method of claim 48 wherein said lymphocyte is a CD4 or CD8 positive cell.

Claim 50 (previously presented): The method of claim 34 wherein said primary cell of the hematopoietic system or hematopoietic stem cell is a CD34 positive cell or a precursor thereof.

Claim 51 (previously presented): The method of claim 34 wherein said primary cell of the hematopoietic system is a human hematopoietic stem cell or a precursor thereof.

Claim 52 (previously presented): The method of claim 34 wherein said at least one cell surface binding molecule comprises a molecule selected from the group consisting of FLT-3 ligand; a TPO ligand; a Kit ligand; an antibody that has the same cell surface binding specificity as FLT-3, TPO, or Kit ligand; a CD3 ligand; a CD28 ligand; a CD25 ligand; a CD71 ligand; a CD69 ligand;

and, an antibody that has the same cell surface binding specificity of CD3, CD25, CD28, CD69 or CD71 ligand.

Claim 53 (previously presented): The method of claim 34 wherein said at least one cell surface binding molecule comprises a molecule selected from the group consisting of FLT-3 ligand, TPO ligand and Kit ligand or polypeptides or other binding molecules that have the same cell surface binding specificity as FLT-3 ligand, TPO ligand, or Kit ligand.

Claim 54 (previously presented): The method of claim 34 wherein the said primary cell or hematopoietic stem cell is a dendritic cell or a cell capable of differentiating into a dendritic cell.

Claim 55 (previously presented): The method of claim 34 wherein said at least one cell surface binding molecule is selected from the group of compositions comprising a CD34, a CD3, a CD28, a GM-CSF, an IL-4, a TNF-alpha; a GM-CSF; an interferon-alpha; and an antibody or other binding molecule that has the same cell surface binding specificity as CD34, CD3, CD28, GM-CSF, IL-4, and TNF-alpha, GM-CSF or interferon-alpha.

Claim 56 (currently amended): The method of claim 34 wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 antibodies and cell surface binding fragments thereof, CD28 antibodies and cell surface binding fragments thereof, combinations of said antibodies and cell surface binding fragments thereof, and binding molecules that have the same cell surface binding specificities as the antibodies,

and optionally the least one cell surface binding molecule comprises at least two of the cell surface binding molecules [[are]] immobilized on a bead or a surface.

Claim 57 (previously presented): The method of claim 56 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 antibodies immobilized on coated beads.

Claim 58 (previously presented): The method of claim 34 further comprising culturing the primary cells or hematopoietic stem cells under conditions conducive to growth and/or proliferation.

Claim 59 (previously presented): The method of claim 58 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

Claim 60 (previously presented): The method of claim 59 wherein said cytokine is interleukin-2.

Claim 61 (previously presented): The method of claim 58 wherein said culturing is for about seven days.

Claim 62 (previously presented): The method of claim 58 wherein said culturing is for about 14 days.

Claim 63 (previously presented): The method of claim 34 wherein said contacting the primary cells or hematopoietic stem cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

Claim 64 (previously presented): The method of claim 34 wherein the lentiviral vector is present at an MOI of less than about 500.

Claim 65 (canceled)

Claim 66 (previously presented): The method of claim 34 wherein said contacting occurs *ex vivo*.

Claim 67 (previously presented): The method of claim 34 wherein said lentiviral vector is derived from a human immunodeficiency virus (HIV), wherein optionally the HIV is HIV-1 or HIV-2.

Claim 68 (previously presented): The method of claim 34 wherein said lentiviral vector is a chimeric vector comprising HIV-1 and HIV-2 sequences.

Claim 69 (previously presented): The method of claim 1 or claim 34, wherein greater than 80%, 85%, 89%, 90%, 91%, 92%, 93%, 94% or 95% of the cells are stably transduced after about 14 days.

Claim 70 (previously presented): The method of claim 34 wherein the individual is infected with a human immunodeficiency virus (HIV), wherein optionally the HIV is HIV-1 or HIV-2.

Claim 71 (previously presented): The method of claim 1 or claim 34, wherein the primary cells or hematopoietic stem cells isolated from the HIV-infected individual are pre-stimulated with at least one cell surface binding molecule, and optionally the primary cells or hematopoietic stem cells are pre-stimulated with the at least one cell surface binding molecule within twenty four (24) hours prior to simultaneously contacting the primary cells or hematopoietic stem cells *in vitro* or *ex vivo* with the lentiviral vector and the at least one cell surface binding molecule.

Claims 72 to 82 (canceled)

Claim 83 (currently amended): The method of claim 1 or claim 34 [[14]], wherein at least 75% of the cells remain stably transduced after about 14 days.

Claim 84 (previously presented): The method of claim 1, the cell surface binding molecule comprises a polypeptide, a lipid, a nucleic acid, a carbohydrate or an ion.

CONT'D THERAPY

Claim 85 (currently amended): The method of claim 1 or claim 34 [[14]], further comprising introducing the transduced cell into a living subject.

Claim 86 (currently amended): The method of claim 1 or claim 34 [[14]], further comprising introducing the transduced cell into a tissue or an organ.

GENE THERAPY

Claim 87 (currently amended): The method of claim 1 or claim 34 [[14]], further comprising introducing the transduced cell into a blastocyst. → allow ball of cells in embryo

Claim 88 (previously presented): A method for stable transduction of a cell with a lentiviral vector comprising

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contacting the cell at the same time *in vitro* or *ex vivo* with a lentiviral vector and at least one cell surface binding molecule, wherein the lentiviral vector is pseudotyped, wherein the pseudotyping comprises co-transfecting or co-infecting a packaging cell with both the lentiviral vector genetic material and genetic material encoding at least one envelope protein of another virus or a cell surface molecule,

wherein at least about 75% of the cells are stably transduced after about seven to ten days, or at about 14 days, and optionally at least 75% of the cells remain stably transduced after about 14 days.

Claim 89 (previously presented): The method of claim 88, wherein the lentiviral vector is pseudotyped with a *Rhabdovirus*.

Claim 90 (previously presented): The method of claim 89, wherein the *Rhabdovirus* is a Vesicular Stomatitis Virus envelope G (VSV-G) protein.

Claim 91 (previously presented): The method of claim 88, wherein the transduced cell is an astrocyte, a skin fibroblast, a epithelial cell, a neuron, a dendritic cell, a lymphocyte, a cell associated with the immune response, a vascular endothelial cell, a tumor cell, a tumor vascular endothelial cell, a liver cell, a lung cell, a bone marrow cell, an antigen presenting cell, a stromal cell, an adipocyte, a muscle cell, a pancreatic cell, a kidney cell, an ovum, a spermatocyte, a cell that

contributes to the germ line, an embryonic pluripotential stem cell or a progenitor cell, a blood cell, a non-nucleated cell, a platelet or an erythrocyte.

Claim 92 (currently amended): The method of claim 1 or claim 34 [[14]], wherein the transduced cell is an astrocyte, a skin fibroblast, a epithelial cell, a neuron, a dendritic cell, a lymphocyte, a cell associated with the immune response, a vascular endothelial cell, a tumor cell, a tumor vascular endothelial cell, a liver cell, a lung cell, a bone marrow cell, an antigen presenting cell, a stromal cell, an adipocyte, a muscle cell, a pancreatic cell, a kidney cell, an ovum, a spermatocyte, a cell that contributes to the germ line, an embryonic pluripotential stem cell or a progenitor cell, a blood cell, a non-nucleated cell, a platelet or an erythrocyte.

Claim 93 (currently amended): The method of claim 1, claim 34 [[14]], or claim 88, wherein said at least one cell surface binding molecule comprises at least two cell surface binding molecules selected from the group consisting of an FLT-3 ligand; a TPO ligand Kit ligand; antibodies that have the same cell surface binding specificity as FLT-3, TPO, or Kit ligand; CD3 ligand; a CD28 ligand; a CD25 ligand; a CD71 ligand; a CD69 ligand; and, antibodies that have are the same cell surface binding specificity of CD3, CD25, CD28, CD69 or CD71 ligand~~;~~

~~wherein optionally the immobilized cell surface binding molecule comprise αCD3 and αCD28~~.

Claim 94 (new): The method of claim 1, wherein the at least one cell surface binding molecule comprises a polypeptide, a lipid, a nucleic acid, a carbohydrate or an ion.

Claim 95 (new): The method of claim 34, wherein the at least one cell surface binding molecule comprises a polypeptide, a lipid, a nucleic acid, a carbohydrate or an ion.

Claim 96 (new): The method of claim 88, wherein the at least one cell surface binding molecule comprises a polypeptide, a lipid, a nucleic acid, a carbohydrate or an ion.

Claim 97 (new): The method of claim 93, wherein the at least two cell surface binding molecules comprise immobilized αCD3 and αCD28.

Claim 98 (new): The method of claim 22, wherein the at least one cell surface binding molecule comprises at least two cell surface binding molecules immobilized on a bead or a surface.